Geographic Variation and Adaptation in the Tasmanian Metallic Skink (*Niveoscincus metallicus*)

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ABSTRACT

Geographic Variation and Adaptation in the Tasmanian Metallic Skink (*Niveoscincus metallicus*)

Species occupying broad geographic ranges tend to display reproductive and physiological traits that compensate for environmental constraints. This thesis examines these traits in the metallic skink, *Niveoscincus metallicus*. The metallic skink is the most widespread of all Tasmanian reptiles. It is a small (2-5 g) skink, found throughout Tasmania, and on most of its offshore islands, occurring from sea level to alpine elevations, in a range of habitat types. It also has a limited mainland distribution, in southern Victoria. This thesis provides a detailed examination of thermal biology and life history adaptation within this species.

The thesis is presented in three main sections, each dealing with an important aspect of adaptation on a geographic scale. In the first section (Chapter 3) I examine the phylogenetic history of *N. metallicus* across its entire distributional range, using restriction fragment length polymorphism analysis (12s-16s rRNA gene) and nucleotide sequence divergence information (16s rRNA sequence). These analyses revealed five phylogenetically distinct subtypes of *N. metallicus*, four of which are found within Tasmania. I selected four field sites from within the distributional range of one of these subgroups for a detailed study of inter-population variation. These sites covered the altitudinal range of the species (two high altitude and two low altitude sites). Within each altitude group, sites were chosen to represent open (little tree cover) and closed (complex tree cover) habitats.

The second section of work (Chapters 4 and 5) investigates thermoregulatory adaptation in *N. metallicus*. As part of this work I examined microhabitat occupation for lizards at all four sites. I found that animals at high altitude bask overtly on rock surfaces while those at low altitude are more covert and bask on logs. These differences represent behavioural adaptations to variation in the thermal environment evident between high and low altitude sites.

The importance of acclimatization and genetic adaptation to the thermal physiology and performance of *N. metallicus* was examined in a series of field and laboratory experiments. Preferred body temperatures did not differ between sites. Nevertheless, thermal adaptation does occur in this species. Animals from high altitude tolerate significantly lower environmental temperatures, and can sprint significantly faster at extreme body temperatures. These differences appear to represent genetic adaptation, and thus data from this study support the "labile" view of thermal adaptation.

The third section to this thesis (Chapters 6 and 7) presents an examination of life history adaptation across my four field sites. Life history was found to vary with altitude; however, habitat effects were also evident. High altitude females live significantly longer than do low altitude lizards and mature one year later; however, no variation in adult body size or size at maturity was detected. Relative clutch mass is highest at low altitude; however, trade-offs between offspring size and litter size are evident between low altitude populations. This trade-off appears to result from predatory induced effects. High altitude young are born with significantly larger abdominal fat reserves, a characteristic of some advantage to young which must hibernate within two months of birth. Evidence from this and previous studies on *N. metallicus*, and from research on the sister species *N. ocellatus*, indicates that life history traits are phenotypically plastic, and do not represent evolved adaptations to environmental constraints. An ability to utilize phenotypic life history responses may be of adaptive significance to species, such as *N. metallicus*, occupying wide geographic and/or climatic ranges, as it allows these animals to exploit fluctuating environments and adapt life history traits to suit present environmental conditions.

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CONTENTS

CHAPTER ONE

General Introduction

1.1 Introduction

Ectotherms living over broad geographical or altitudinal ranges are presented with significant challenges arising from variation in a number of factors including thermal environment, habitat, food availability, intra and inter specific competition and predation (Smith, 1996, 1998; Schneider *et al.*, 1999). Therefore, reptiles living over such broad areas often display reproductive, physiological and behavioural traits that compensate for environmental constraints (Stearns, 1976; Dunham *et al.*, 1988; Smith *et al.*, 1993; Niewiarowski, 1994).

One factor often examined in studies of geographic adaptation is thermal environment. The interaction between reptiles and their thermal environment has long interested ecologists (e.g. Cowles and Bogert 1944; Bogert, 1949). In particular, the responses of ectotherms to the thermal problems imposed by high altitude have received considerable attention for many years (e.g. Swan, 1952; Burns, 1970). Both altitude and latitude have been shown to significantly influence thermal ecology and life history adaptation in a number of reptile species from several families (Van Damme *et al.*, 1989; Smith *et al.*, 1993; Forsman and Shine, 1995; Rohr, 1997; Schwarzkopf, 1998; Qualls and Shine, 1998; Abell, 1999). Nevertheless, knowledge about causal mechanisms responsible for inter and intra specific variation in these characteristics is poor, and data for many groups, including skinks, are rare (Dunham *et al.*, 1988; Vitt and Pianka, 1994). Our knowledge of the ecological and evolutionary significance of life history diversity and thermal variability in lizards is based primarily on studies from North American and European species (e.g. Crowley, 1985; Sinervo and Adolph, 1989; Sinervo, 1990a, 1990b; Gillis, 1991; Adolph and Porter, 1993; 1996; Smith *et al.*, 1993). The Scincidae are particularly poorly known (Dunham *et al.*, 1988, Vitt and Pianka, 1994), considering their status as the world's largest and most diverse lizard family (Greer, 1989; Hutchinson, 1993). The high degree of phylogenetic conservatism in reptiles (Stearns, 1984; Dunham *et al.*, 1988) means that independent data sets are required for species from other taxa, to fully understand

adaptation to environmental variability (Forsman and Shine, 1995). Few Australian skinks have provided any data on geographic variability in reproductive life history (Hutchinson, 1993).

A basic premise underlying most hypotheses dealing with the evolution of life histories is that variation is constrained by trade-offs between life history traits (Smith, 1991; Roff, 1992; Niewiarowski and Dunham, 1994). This is believed to occur because natural selection is unable to maximise all life history traits simultaneously (Partridge and Sibly, 1991). Two particular types of trade-offs are recognized: physiological and evolutionary (Smith, 1991, Stearns, 1992). The first arises because a limitation in resource availability means that increasing allocation to one function (i.e. growth, reproduction or storage) can only occur at the expense of another (Cody, 1966; Smith, 1991; Streams, 1992). Evolutionary trade-offs occur when an increase in the fitness of one life-history trait results in a decrease in the fitness of another. Thus evolutionary trade-offs deal with the genetic basis of traits and how these respond to selection (Pamula, 1997) and can therefore include a component of plasticity in the expression of the phenotype. Stearns (1989; 1992) has identified at least forty five major life history trade offs. Of these, the most studied are those between current reproduction and future reproduction, current reproduction and survival, reproduction and growth, reproduction and condition, and number and quality of offspring.

Geographic variation in life history phenotypes between populations of a widely distributed species is often assumed to reflect genetic divergence caused by natural selection. However, the relative importances of genetic and environmental sources of phenotypic variation are rarely determined. Proximate sources are those that cause variation in phenotype arising from the same genotype, while evolutionary sources imply that genetic differences are responsible for phenotypic variation (Niewiarowski, 1994). Distinguishing between phenotypic plasticity in life history induced by environmental variability and that induced by genetic divergence is fundamental to interpreting the ecological and evolutionary significance of geographic variation (Niewiarowski and Roosenberg, 1993). This is because intra-specific variability may be a result of physiological or developmental responses to environmental conditions (Stearns and Sage, 1980) rather than adaptation to local selective pressures (Stearns, 1980).

One of the most obvious proximate factors with the potential to influence life history in reptiles is temperature. Body temperatures experienced by an organism directly affect individual growth (Huey, 1982), defensive behaviour (Hertz *et al.*, 1982; Losos, 1988) and reproduction (Andrews *et al.*, 1997). The physiological effects of body temperature can also affect energy intake (Avery *et al.*, 1982) and assimilation (Beaupre *et al.*, 1993). Similarly the thermal dependence of sprinting and stamina may influence an animal's ability to avoid predators (Bennett, 1980; Christian and Tracy, 1981; Hertz *et al.*, 1988; Mautz *et al.*, 1992). Nevertheless the effects of environmental temperature variation are complicated by the fact that many lizard species thermoregulate to maintain a relatively constant body temperature, over a wide range of environmental temperatures (Adolph and Porter, 1993). Recently attention has also focussed on the effect of temperature on activity period (Grant and Dunham, 1988; Adolph and Porter, 1993, 1996). Thermoregulatory behaviour is generally considered to be adaptive; however, there are a number of associated costs for an animal in terms of time, energy (increased metabolism associated with increased body temperature), potential competition for microhabitats, or increased predation (Huey and Slatkin, 1976; Avery *et al.*, 1982; Huey, 1982; Grant, 1990). Lizards may therefore also be faced with potential thermoregulatory trade-offs affecting energy assimilation and survival, all of which can significantly impact on several life history traits, including growth and reproductive output (Pamula, 1997). Thus thermal ecology and life history are interrelated and thermal adaptation to environmental variability can represent a key determinant of energy assimilation and ultimately total reproductive effort.

While temperature represents one of the most important proximate factors influencing life history, other factors also affect reptile growth and reproduction. Resource availability, for example, has been shown to significantly influence many life history traits (Andrews, 1982; Ballinger, 1983), including growth rate (Dunham, 1978; Guyer, 1988; Smith and Ballinger, 1994a, 1994b; Wapstra; 1998), survivorship (Ballinger, 1984; Smith and Ballinger, 1994c), clutch size and mass (Ballinger, 1977; Seigel and Ford, 1991; Swain and Jones, 2000b), and age at first reproduction (Ford and Seigel, 1994). Precipitation has also been shown to influence life history traits on a temporal basis (Stamps and Tanaka, 1981; Seigel and Fitch, 1985; Smith *et al.*, 1995). However, this decrease in water availability has generally been correlated with a reduction in prey availability. Tail loss and social status have also been shown to represent significant proximate life history forces (Bauwens, 1981; Andrews, 1982; Arnold, 1988; Chapple, 2000).

While proximate factors represent significant sources of variation in reptile growth and life history (Hudson, 1997), a large body of evidence also indicates that evolutionary sources are also significant. Controlled common garden experiments have revealed variation among species and populations in growth rate and many reproductive traits (Ferguson and Brockman, 1980; Sinervo and Adolph, 1989; Ferguson and Talent, 1993; Smith *et al.*, 1994). Transplant experiments have also indicated that in some species genetic sources may in part explain variation in growth rate among populations (Ballinger, 1979; Niewiarowski and Roosenburg, 1993; Niewiarowski, 1995). This type of work has, however, been largely restricted to a single group, the sceloporine lizards, and other, phylogenetically distinct, taxa are likely to display somewhat different patterns of life history adaptation.

Thus, while it may be agreed that both proximate and genetic sources of life history variation are of significant importance in squamates, the relative importance of these two sources, in a broad range of taxa, is unclear (Niewiarowski, 1994; Hudson, 1997). Even when differences in life history traits are identified and can be related to current selection pressures, absence of population-level phylogenetic information can confound interpretation of the historical role of natural selection in producing these differences (Niewiarowski, 1994). Reptiles and specifically lizards have proven to be ideal subjects for studies of intra-specific adaptation of life history, and for examinations of the relative importance of genetic and environmental sources of variability. This partly reflects the relative ease with which many of their life history traits can be quantified (Ballinger, 1983; Schwarzkopf, 1994). This study examines variation in thermal adaptations and life history between populations of the Tasmanian metallic skink *Niveoscincus metallicus*. Data are collected from one phylogenetic sub-group, to remove the influence of historical isolation from the actions of diversifying selection across environmental gradients.

1.2 Study species

1.2.1 Tasmanian reptiles

The Tasmanian terrestrial reptile fauna has been described as relatively impoverished (Heatwole, 1976; Hutchinson *et al.*, 1988). There is a total of twenty species from three families: the Scincidae (16 species), the Agamidae (1 species) and the Elapidae (3 species). Due to its southern location the majority of the Tasmanian reptile fauna consists of viviparous species. There are only three egg laying species: the agamid, *Tympanocryptus diemensis*, and the skinks *Bassiana duperryii* and *Lampropholis delicata*. These species depend on warm microclimates for successful incubation of eggs. Thus their distribution tends to be limited to the warmest parts of the state, although *Lampropholis delicata* is successful as far south as Hobart (Wapstra and Wapstra, 1986; Brereton *et al.*, 1996, personal observation). Interestingly, a fourth species, *Lerista bougainvillii*, is viviparous in Tasmania and oviparous in some mainland populations (Greer, 1989; Qualls and Shine, 1995).

1.2.2 The genus *Niveoscincus* ('snow skinks')

Members of the genus *Niveoscincus* are small to medium sized lygosome lizards, characterized by the possession of a well developed prefrontal shield, paired or fused frontoparietals, and a moveable lower eyelid with a moderate to large transparent palebral disc (Hutchinson *et al*,. 1989). All members of the genus also possess well-developed pentadactyl limbs and a viviparous mode of reproduction (Hutchinson *et al.*, 1989, 1990).

Most species of *Niveoscincus* are entirely restricted to the island state of Tasmania and its adjacent offshore islands. The only exceptions are the species *N. coventryi*, found only on the mainland, and *N. metallicus*, whose range extends from Tasmania into southern and eastern Victoria. Of the six endemic species, five have restricted and/or isolated habitat requirements (Rawlinson, 1975; Hutchinson *et al.*, 1989, 1990; Melville and Swain, 1999b): these are *N. greeni*, *N. microlepidotus*, *N. orocryptus*, *N. pretiosus* and *N. palfreymani*. The two remaining Tasmanian species, *N. metallicus* and *N. ocellatus* have widespread distributions. They are a closely related monophyletic group (Hutchinson *et al.*, 1990; Hutchinson and Schwaner, 1991; Melville and Swain, 2000b) that until recently

were included in the genus *Leiolopisma* (Hutchinson *et al.*, 1990). The entire genus is viviparous, a characteristic that is shared only by *Pseudemoia* among Australian eugongylus lizards. Viviparity is generally regarded as an adaptive characteristic in species inhabiting cool climates (Shine and Bull, 1979; Qualls, 1997).

Three phylogenetically distinct ecomorphs have been identified in this genus: ground-dwelling; semi-arboreal; and saxicolous (Melville, 1998; Melville and Swain, 1999b, 2000a, 2000b). Ground dwelling species (*N. metallicus* and *N. coventryi*) are characterized by relatively short limbs and long inter-limb lengths. The fact that both non-endemic species are ground dwelling suggests an evolutionary scenario starting with colonisation of Tasmania by a "metallicus-like" species. The ability to climb appears to have evolved in the semi-arboreal species (i.e. *N. pretiosus*) and has involved a decrease in body size and an increase in relative leg length. The saxicolous species have evolved the ability to jump, with an increase in relative leg length. Saxicolous species can be further divided into heath/rock-dwelling (*N. orocryptus*, *N. microlepidotus*) and saxicolous specialists (*N. greeni*) (Melville, 1998; Melville and Swain, 2000a, 2000b).

All species of *Niveoscincus* are diurnal shuttling heliotherms, maintaining active body temperatures often well above ambient temperature by alternating between basking and moving to shaded areas (Rawlinson, 1974, 1975; Hutchinson *et al.*, 1989). They all display a bimodal daily activity pattern on sunny days, with basking in the morning and afternoon, and foraging /shuttling in the middle of the day (Wapstra, 1993; Melville and Swain, 1997a). During cooler periods basking may be extended and activity patterns may become unimodal. One species, *N. microlepidotus*, has also been shown to display thigmothermic behaviour, as a method of extending activity during cooler periods in the alpine zone (Melville and Swain, 1997a).

Eleven classes of reproductive cycles have been identified in Australian reptiles, with three being known to occur in temperate Australian skinks (Taylor, 1985; Heatwole and Taylor, 1987). Most *Niveoscincus* species for which reproductive cycles are known exhibit a type II cycle, which is characterized by autumn spermatogenesis and mating, and spring ovulation (Pengilley, 1972; Rawlinson, 1974; 1975; Hutchinson and Donnellan, 1988, 1992; Hutchinson *et al.*, 1988, 1989); however, in these species a second mating also occurs in spring. Interestingly high altitude *N. microlepidotus* display a unique biannual

reproductive cycle with birth occurring in spring (Olsson and Shine, 1999). *Niveoscincus* have played an important role in our understanding of squamate reproduction, and specifically placentation and maternal-foetal nutrient transfer. Weekes (1935) recognised three morphotypes of chorioallantoic placenta in viviparous squarmates, and a fourth was recently described by Blackburn (1993) in New World *Mabuya* skinks. The majority of skinks display Type 1 placentation (Yaron, 1985; Blackburn, 1993). However, a more complex placental morphology, Type II, was described by Weekes (1930, 1935) from mid-gestational embryos of *N. ocellatus*. Type II placentation has also been described in other *Niveoscincus* species, including *N. metallicus* (Stewart and Thompson (1994); however, the authors noted some structural differences between this species and the placental arrangements described by Weekes (1930) in *N. ocellatus*. Recently the placental structure of *N. ocellatus* was reexamined and found to be of a type III structure (M. Thompson, pers com). However, type II placentation appears most common in this genus, with significant interspecific variation in complexity present. Type III placentation has been described mainly in *Pseudemoia* species; however the possibility exists that it is also evident in *N. coventryi* (Hudson, 1997) as well as *N. ocellatus*.

Until quite recently the only major work on the snow skinks was provided by Rawlinson (1974). However, during the past decade a significant amount of research on phylogenetic relationships (Hutchinson *et al.*, 1988, 1990; Hutchinson and Schwaner, 1991; Melville and Swain, 1998; Melville and Swain, 2000a), distribution, ecology, behaviour (Green and Rainbird, 1993; Wapstra and Swain, 1996; Melville and Swain 1997a,b, 1999a,b, 2000b; Olsson *et al.*, 2000), physiology, life history and reproductive ecology (Swain and Jones, 1994, 1997, 2000a, 2000b; Jones and Swain, 1996, 2000; Hudson, 1997; Jones *et al.*, 1997, 1998; Olsson and Shine, 1998a,b, 1999; Wapstra *et al.*, 1999; Thompson *et al.*, 1999a; Olsson *et al.*, 2000; Wapstra, 2000; Wapstra and Swain, in press a,b) has been conducted.

1.2.3 *Niveoscincus metallicus*

The metallic skink is the most widely distributed and ubiquitous member of the genus with its distribution extending across the majority of Tasmania and its coastal islands, Bass Strait islands and into south-eastern Victoria. The species is predominantly ground dwelling, inhabiting a diverse range of natural habitats including dry sclerophyll

woodlands, grasslands, sub-alpine woodland and heathland (Melville and Swain, 1999b). It has also been reported from intertidal areas on the south west coast of Tasmania (Schulz and Kristensen, 1994); however, these authors may have misidentified *N. pretiosus*. The species is also well adapted to disturbed habitats and is commonly found in large abundance in suburban gardens and in roadside vegetation. *Niveoscincus metallicus* is a relatively cryptic species that primarily occupies habitats characterized by significant shade, dense vegetation and litter, fallen logs or rocks. It is an active forager that eats predominantly arthropod prey and some vegetative structures. While the species appears to occur primarily in areas with extensive arboreal vegetation (Rawlinson, 1974), it remains largely confined to the ground, only climbing rocks or logs to bask (Melville and Swain, 1997a, 1999b, 2000b). As with other members of the genus, it is a shuttling heliotherm with an active diurnal activity pattern.

Niveoscincus metallicus is one of the smaller members of the genus with an adult snout-vent length (SVL) ranging from 45 mm to 65 mm. Animals weigh between 2 g and 5 g, and have a long tail that can extend to 140% of the SVL (Chapple, 2000). Its color markings are highly variable, ranging from brown to bark black on its dorsal surface, with vertebral strips or flecks and a mid-lateral streak. Sexual variation is also present in relation to morphology and coloration. Males tend to attain similar SVLs but have a relatively smaller inter-limb length (Melville, 1994). They also have a more pronounced head, and more intensive ventral coloration. The significance of this variation in color is unknown (Swain and Jones, 1994).

The species in an annual breeder with clutch sizes ranging from 1 to 8 but generally between 2 and 5. (Mean: 3 to 4 depending on population) (Jones and Swain, 1996; my unpublished data). Young are born fully developed and totally independent with a SVL ranging between 15 mm and 25 mm. Parental care is absent (Bennett, 1997). *Niveoscincus metallicus* exhibits both lecithotrophy and placentotrophy (Jones *et al.*, 1998; Thompson *et al.*, 1999), and the presence of facultative placentotrophy has also been demonstrated (Swain and Jones, 2000a,b).

All *Niveoscincus* species exhibit an asynchronous type II reproductive cycle (Swain and Jones, 1994; Jones and Swain, 1996; Hudson, 1997). Vitellogenesis begins in late summer (February to March), first mating occurs in April with females storing sperm over winter

until ovulation and fertilisation occurs in spring (September to November) following emergence (Swain and Jones, 1994; Jones and Swain, 1996; personal observations). A second mating often occurs in early spring (Swain and Jones, 1994). In all cases reproductive events are delayed in high altitude populations and overwintering begins earlier at high altitude (Figure 1.1). Gestation is completed by late December to late February depending on environmental conditions.

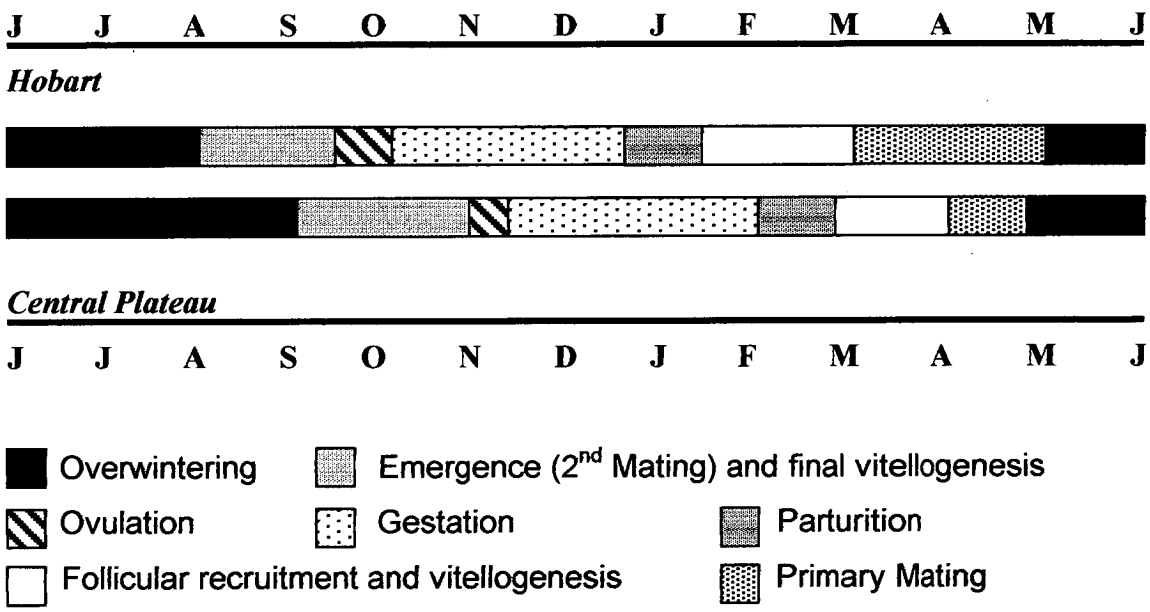

Figure 1.1 Asynchronous timing of female reproductive events for *N. metallicus* populations at Hobart and the Central Plateau (Jones and Swain 1996, personal observations).

Due to its ubiquitous occurrence throughout Tasmania, *N. metallicus* co-exists with almost all other members of the genus, including most species with limited distributions. However, it is distinguished from other species by the presence of large mid body scales (22-29 rows) and possession of a long slender body with relatively small limbs. This second characteristic allows it to co-exist with other members of the genus, which are largely saxicolous or arboreal in nature (Melville and Swain, 2000b). The fact that several aspects of the biology and ecology of *N. metallicus* have been extensively studied (Melville, 1994, 1998; Swain and Jones, 1994, 1997, 2000a, 2000b; Jones and Swain, 1996; Melville and Swain, 1997a,b, 1999b, 2000b; Jones *et al.*; 1998; Chapple, 2000), along with its extensive geographic range makes it an ideal model species to examine geographic adaptation to environmental variability.

1.3 Overview of this thesis and major research objectives

This thesis examines adaptation in *Niveoscincus metallicus* across a range of locations. The experimental component of the thesis can be broadly divided into three main sections. The first of these is an examination of genetic divergence between populations across the entire range of the species (Chapter 3). From this work a number of sites were selected, based on minimum divergence, to examine adaptation to environmental extremes. The second section of this thesis (Chapters 4 and 5) examines adaptation in thermal ecology in *N. metallicus*. Chapter 4 studies variation in habitat selection as a method of adaptation across a number of habitats. Following this Chapter 5 examines the thermal ecology of *N. metallicus*, specifically to determine if this species uses physiological or genetic adaptation to enable it to successfully exploit habitats with vastly differing thermal characteristics. The final experimental section of this thesis examines life history in *N. metallicus*. Chapter 6 describes variability in offspring phenotype and clutch characteristics from a number of sites, while Chapter 7 specifically focusses on age-size relationships across the altitudinal range of the species. The final chapter of the thesis (Chapter 8) provides an overview of inter-population variation and adaptation in this species and evaluates future research opportunities.

The primary aim of this thesis is to investigate adaptation in a ubiquitous species of skink found throughout Tasmania and in a large area of mainland Victoria from sea level to sub-alpine elevations. The research was conducted to evaluate the effects of both altitude and habitat type on the thermal biology and reproductive life history of, arguably, the most successful member of the genus *Niveoscincus*. The objectives of my study were to:

1. examine variation in genotype between populations of *N. metallicus* and to determine the possible series of events which may have lead to the present day distribution of genetically distinct sub-groups of the species (Chapter 3).

2. quantify microhabitat occupation in *N. metallicus* and to examine its importance in the adaptation of this species to environmental variability (Chapter 4). The possible role of factors such as competition and predation, that may influence microhabitat selection, are also discussed.

3. examine variation in thermoregulatory behaviour between populations exposed to differing habitat features and climatic conditions (Chapter 5).

4. evaluate the importance of physiological acclimation and genetic adaptation, to the thermal biology of *N. metallicus* in populations covering the altitudinal range of the species (Chapter 5).

5. measure variation in offspring and clutch characteristics on an annual and geographic basis in *N. metallicus* and to evaluate the influence of environmental variability and genetic adaptation on these components of life history (Chapter 6).

6. examine age-size relations in *N. metallicus* in order to determine if age at maturity, longevity and growth are affected by location, and to examine whether variation in thermal opportunity alone can account for any observed differences between populations.

CHAPTER TWO

General Materials and Methods

2.1 Introduction

Tasmania, the most southerly and only island state of Australia, is isolated some 200 km south of mainland Victoria by Bass Strait. Situated at 40 to 43.5° south and 140 to 150° east, the State experiences a cool to cold temperate climate characterized by unpredictable weather patterns.

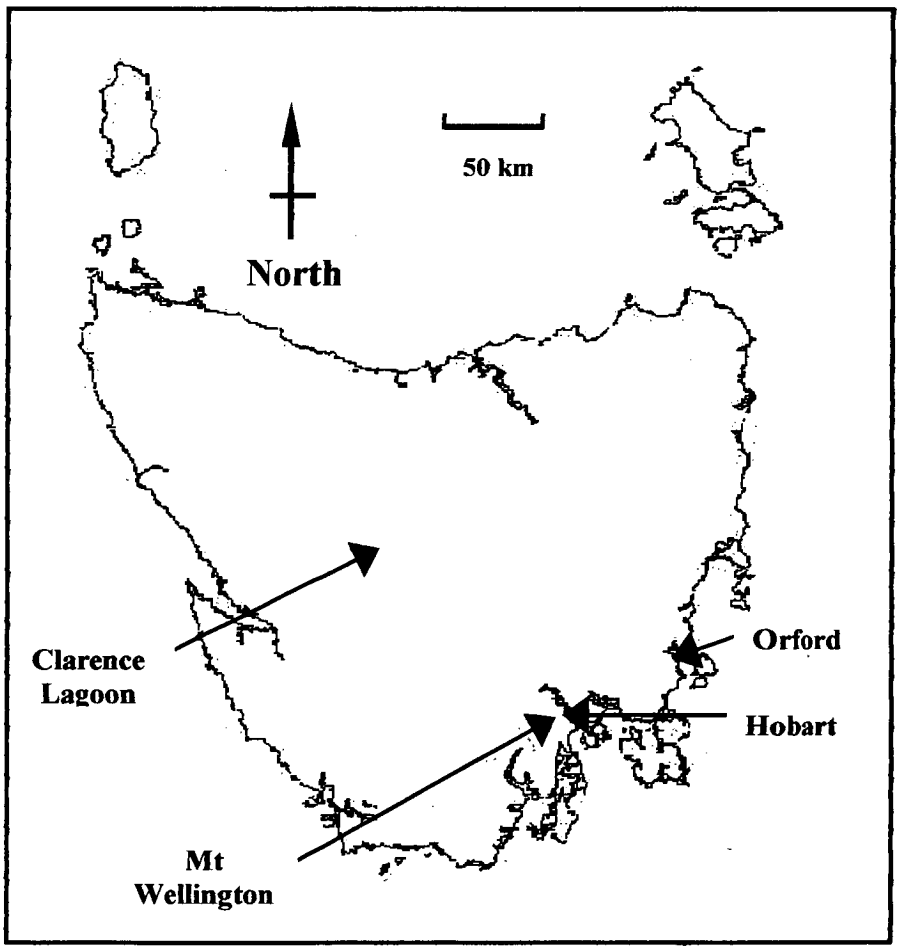

Figure 2.1 Location of Tasmanian field sites used in Chapters 4 to 7. The range of *N. metallicus* is continuous throughout the island. Sites on the right are low altitude (below 150 m) and sites on the left are high altitude (approximately 1000 m).

This investigation has three main sub-components. The first, a genetic examination of variation across the range of *N. metallicus*, utilized a number of field sites, not all of which were in Tasmania. These sites are considered in Chapter 3. The remaining two components of the thesis examine in more detail thermal ecology and life history adaptation in this species, and, only four field sites, covering the altitudinal limits of *N. metallicus* were used. All these sites are from within the Type I populations identified through an analysis of population genetics and all are located in Tasmania itself (Chapter 3). Two sites, Mt Wellington and Clarence Lagoon, are found at high altitude (approximately 1000 meters a.s.l). The first is an open sub alpine habitat, while the second is an extensively forested and closed environment. The remaining sites, Hobart and Orford, are low altitude sites (below 150 meters a.s.l). Again one, Orford, while forested, is an open, exposed habitat, while the second, Hobart, represents a forested and closed environment. The location of study sites is shown in Figure 2.1. The sites are described more fully below.

2.2 Description of study sites

2.2.1 Clarence Lagoon

Clarence Lagoon (146°19' E, 42°04' S) is situated on the southern margin of the Central Plateau World Heritage Area. The site is dominated by a small glacial lake at approximately 1000 m a.s.l., formed as a result of a glacial moraine. The site is approximately 10 km from Lake St Clair (closest weather station situated at 735 m a.s.l.,). The southern shore of the Lagoon is surrounded by a narrow band of boulders, formed as a result of glacial movement. The land around the Lagoon is characterized by poor thin soil with boulders breaking through the surface. At the Lagoon the site is exposed; however, as the land drops away steeply to the south, conditions are more sheltered. All areas support forest, but further away from the Lagoon, the habitat is more complex with rain forest elements.

Vegetation at the Lagoon edge is tall sub-alpine forest (Figure 2.2). The canopy is of medium height (10 meters) and allows significant amounts of sunlight to enter. The understorey is patchy in most areas, but can be substantial. Two forest type are evident further away from the Lagoon. The first is sclerophyll forest and is similar in structure to

forest at the Lagoon edge. The second forest type is mixed forest. This habitat is complex, in both understorey and canopy. Throughout all habitat types, rocks emerge from the thin soil layer. A number of plant species are common at the site. Those dominant in sub alpine and sclerophyll areas include *Richea sprengelioides*, *Telopea truncata*, *Boronia citiodora*, *Banksia marginata* and a number of eucalyptus species.

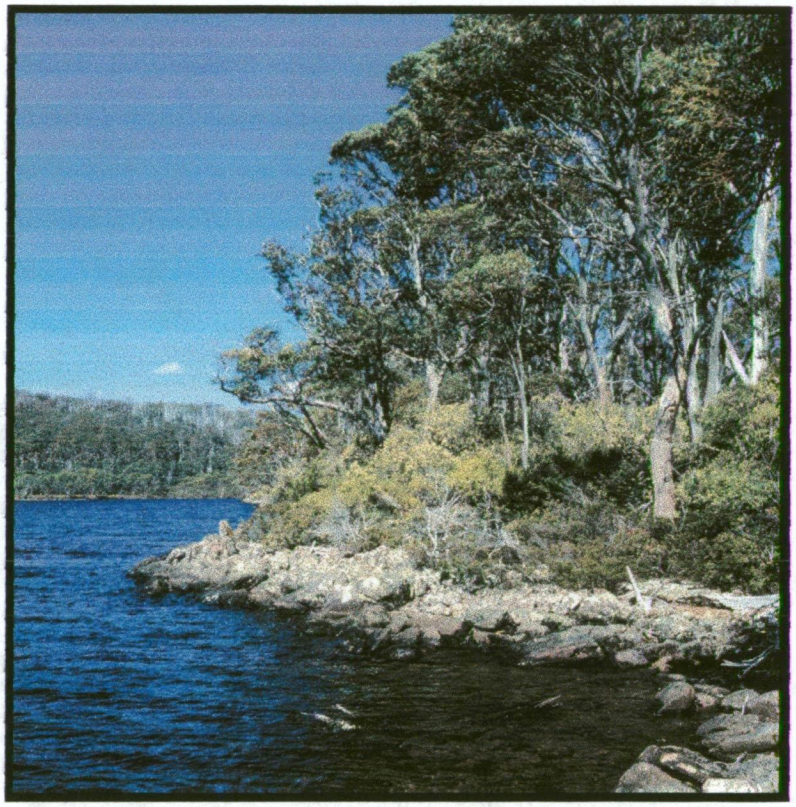

Figure 2.2 Tall dry sclerophyll forest located at the banks of Clarence Lagoon (1000 m a.s.l.). Also evident are exposed boulders around the southern edge of the lagoon. *Niveoscincus metallicus* is most common within the forested habitat but also emerges onto the boulders during early morning and late afternoon.

Niveoscincus metallicus is continues and common throughout all of the above habitat types. A second species *N. pretiosus* is also common at the site, but is restricted to areas of mixed forest with fallen trees. *Niveoscincus ocellatus* is also found in rockier areas, most commonly against the Lagoon shore. *Drysdalia coronoides* (white-lipped snake) and *Notechis ater* (tiger snake) are also found at the site in large numbers.

2.2.2 Mt Wellington

Mt Wellington (147°13'E, 42°53'S) is located in southern Tasmania on the edge of the Derwent River Valley, and sits above the city of Hobart. The study site is located at an altitude of 1080 m, on the eastern face of the mountain near the Big Bend (a feature of the road near the summit of the mountain). The site is a sub-alpine to alpine transition zone (Figure 2.3). Three distinct habitat types are present at the field site: (1) sub-alpine woodland; (2) alpine heath; and (3) boulder fields. *Niveoscincus metallicus* is, however, found only in the first two habitat types and most commonly in the sub-alpine woodland. The species is excluded from true alpine habitat at higher altitudes.

Figure 2.3 Habitat types present at the Mt Wellington field site. The site is situated at 1080 m a.s.l., at the treeline. The three habitat types present at the site, sub-alpine forest, alpine heath and boulder scree, are all represented in the picture. *Niveoscincus metallicus* is most commonly associated with the forest habitat.

The total number of plant species present at this site is somewhat less than at Clarence Lagoon. Two eucalyptus species, *E. amygdaline* and *E. viminalis* form a sparse tree cover of up to 8 m in height. A number of shrub species are also present.

There is a high abundance of reptiles at this site, along with a number of different species. The only other common *Niveoscincus* species present is *N. microlepidotus*; however, *N.*

pretiosus and *N. ocellatus* are also present. Three other reptile species have been observed at the site. These are *Notechis ater* (tiger snake), *Drysdalia coronoides* (white-lipped snake) and *Cyclodomorphus casuarinae* (she-oak skink).

2.2.3 Hobart

The Hobart field site (147° 20'E, 42° 55'S) is situated in the Cascade Reserve area near the base of McRobies Gully and extends north to the lower slopes of Knocklofty Reserve (total area approximately 1 km by 500 m). The area is disturbed by recreational use, encroachment by non-native species, and greater than normal fire frequencies. Soils at the site are a dolerite based duplex typical of eastern and southern Tasmania (Figure 2.4).

Figure 2.4 Tall dry forest located at the Hobart field site (150 m a.s.l.). Two main habitat types, dry sclerophyll forest and grassland, characterize the site. *Niveoscincus metallicus* is found throughout both habitat types.

The study site has two vegetation types: dry sclerophyll forest; and grassland. The forest is typical of the Hobart area with a reasonably open canopy and variable understorey. The

canopy consists of species such as *Eucalyptus obliqua*, *E. amygdalina*, *E. viminalis* and *E. ovata*. The ground is quite rocky with areas of thick bark and fallen leaves around the base of trees. Grassland areas are dominated by exotic species.

A number of reptile species occur at this site. The most common species, besides *N. metallicus* are *N. pretiosus* and *Bassiana duperryi* (Three Lined Skink). Also present are *Pseudemoia entrecasteauxii* (Southern Grass Skink), *Lampropholis delicata* (Delicate Skink), *Tiliqua nigrolutea* (Bluetongue Lizard) and *Tympanocryptus diemensis* (Mountain Dragon). *Notechis ater* and *Drysdalia coronoides* (elapid snakes) were also both observed at the site.

2.2.4 Orford

This site is located approximately 2 km inland from the small township of Orford on the East Coast of Tasmania (147° 51' E, 42° 34' S). The site has a very high fire frequency, being most recently burned during 1995. The area is warm and relatively dry, with high temperatures during the summer months. Low hills of weathered dolerite, with a shallow soil and extensive areas of scree dominate the site.

The vegetation at the site is dry sclerophyll with a very open canopy and a very sparse understorey. However, in damper gullies the forest changes to a wet sclerophyll with a denser understorey. Rock outcrops are common throughout. The field site covered all habitat types, but the majority of the area was dry sclerophyll, and this habitat tended to be favored by *N. metallicus*. Vegetation makeup was very complex, reflecting the high fire frequency and invasion by exotic species. *Eucalyptus pulchella*, *E. viminalis* and *E. amygdalina* were the dominant tree species.

The study site had a high diversity of reptile species. The most common species were *N. metallicus*, *N. ocellatus*, *N. pretiosus* and *Egernia whitii* (White's Skink). Also present were *Psuedemoia entrecasteauxii*, *Cyclodomorphus casuarinae* (Sheoak Lizard), *Tympanocryptis diemensis* (Eastern Mountain Dragon), *Tiliqua nigrolutea* and *Bassiana duperryi*. Both *Notechis ater* and *Drysdalia coronoides* were also captured at the site.

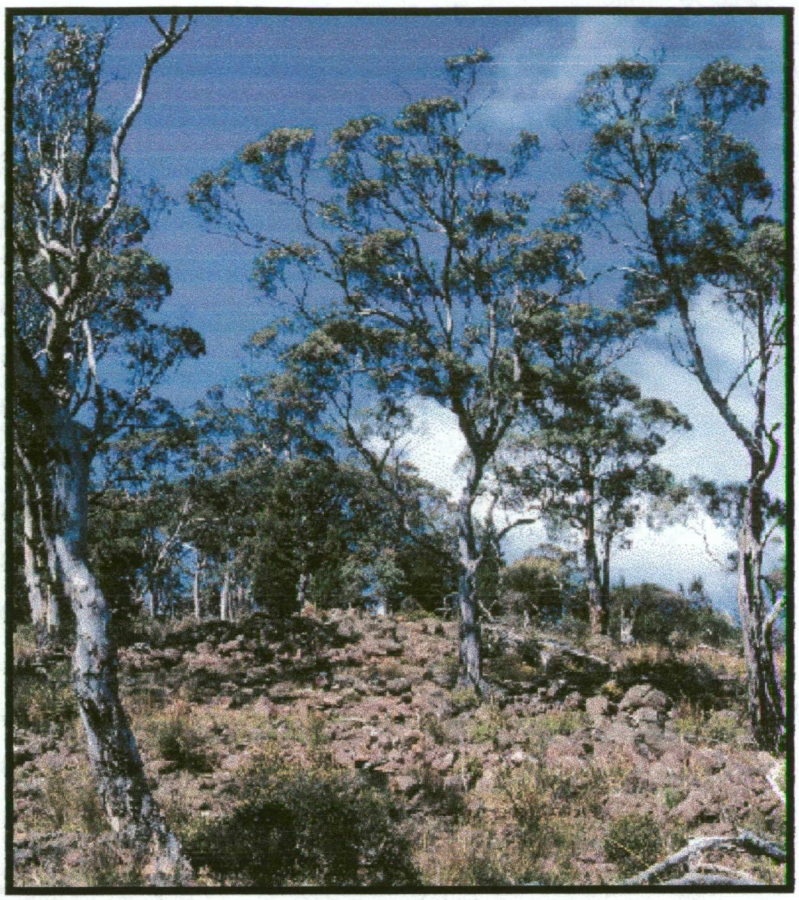

Figure 2.5 Open dry forest located at the Orford field site (50 m a.s.l.). Two main habitat types, dry sclerophyll forest and boulder scree, characterize the site. However, wetter and more complex forest is found in river gullies. *Niveoscincus metallicus* is found throughout most of the available habitat types but is uncommon on scree.

2.3 Comparison of climatic conditions between study sites

All study sites differ substantially in terms of thermal conditions and general weather features. The Orford and Hobart study sites are low altitude sites and are classified as cool temperate. Conversely the Mt Wellington and Clarence Lagoon sites are high altitude and can be described as cold temperate (Rawlinson, 1974). Climatic data for each site was obtained from the Bureau of Meteorology web site (www.bom.gov.au). Table 2.1 identifies the location and elevation of the weather station nearest to each field site.

Table 2.1 Details of the closest weather stations to sites used in this investigation. All stations are at a similar altitude and aspect to sites of data collection. Also shown is the direct distance between the weather station and the field site it was used to describe.

Study Site	Station	Distance between site and station	Bureau site number	Latitude	Longitude	Elevation	Dates of data collection
Clarence Lagoon	Lake St Clair	11 km	096015	42° 10' S	146° 22' E	735.0 m	1937-1989
Mt Wellington	Mt Wellington (Summit)	2 km	094087	42° 90' S	147° 24' E	1260.5 m	1961-1996
Hobart	Hobart (Ellerslie Road)	3 km	094029	42° 89'	147° 33' E	50.5 m	1882-1996
Orford	Orford (Town Post Office)	2 km	092027	42° 55' S	147° 88' E	15.0 m	1951-1996

2.3.1 Temperature variation between sites

All sites display distinct thermal characteristics. Low altitude sites are warmer than high altitude sites throughout the year, both for maximum and minimum temperatures (Figures 2.6 and 2.7). Hobart appears generally to have lower maximum temperatures for both summer and winter than Orford; the reverse is true for minimum winter temperatures. Despite these differences the two sites are very similar. The Lake St Clair station experiences warmer temperatures than the Mt Wellington station. While Mt Wellington would be expected to be slightly colder than Clarence Lagoon, the differences shown in Figures 2.6 and 2.7 also reflect the fact that the Lake St Clair station in situated at a lower altitude than the Clarence Lagoon site (approximately 200 m) and the Mt Wellington station is above the Mt Wellington field site (also 200 m). Thus, these two sites are in fact thermally more similar than the figures suggest. Maximum air temperatures differ most between sites during winter months, most notably between Clarence Lagoon and the two low altitude sites. However, minimum temperatures remain lower at high altitude by a relatively constant amount throughout the year.

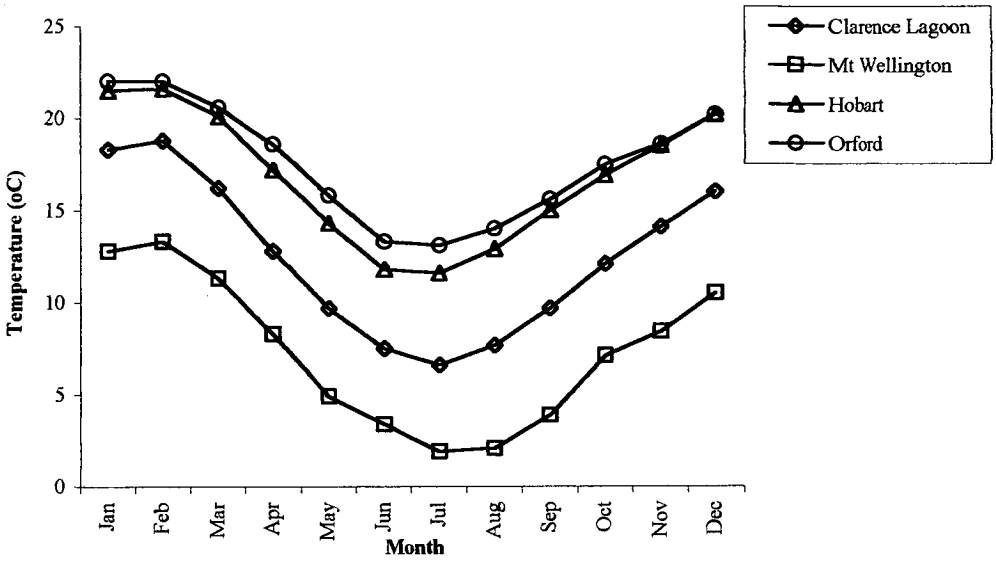

Figure 2.6 Mean monthly maximum air temperatures (°C) for periods indicated in Table 2.1 for the Clarence Lagoon, Mt Wellington, Hobart and Orford areas.

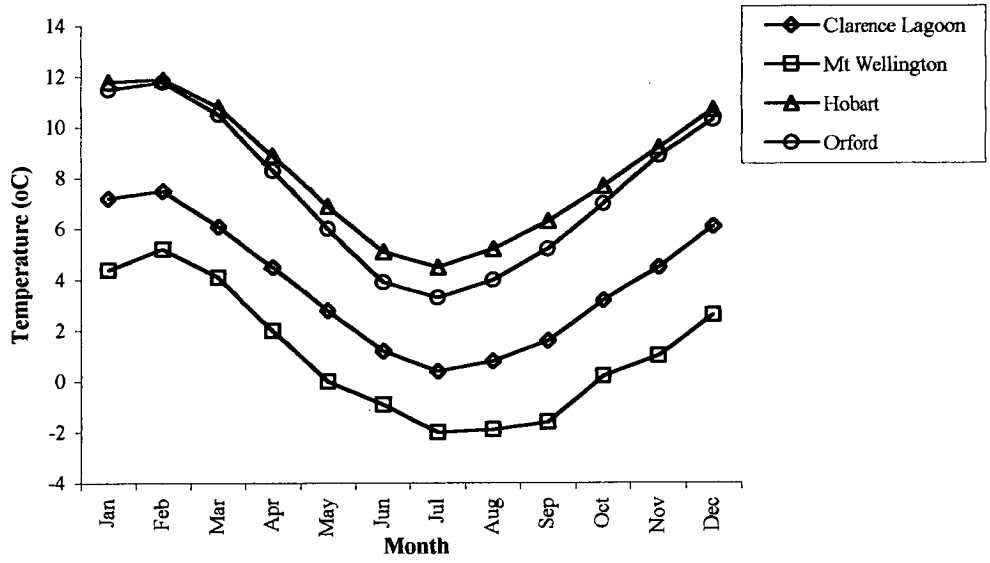

Figure 2.6 Mean monthly minimum air temperatures (°C) for periods indicated in Table 2.1 for the Clarence Lagoon, Mt Wellington, Hobart and Orford areas.

2.3.2 Precipitation, cloud cover and available sun light

Substantial differences exist between sites in terms of precipitation and sunlight availability. Both high altitude sites receive more rain than the low altitude sites; however, Clarence Lagoon receives substantially more rain than any of the other sites (Figure 2.7). This is especially noticeable during late autumn, winter and spring. During summer, this difference is very much reduced. Mean number of rain days also reflects the poorer environmental conditions experienced by animals living at high altitude (Figure 2.8). Rain days appear to remain high at high altitude throughout the entire year, although less rain falls during summer at all sites. During June there is a decrease in rainfall at both high altitude sites; however, this lasts only for that single month.

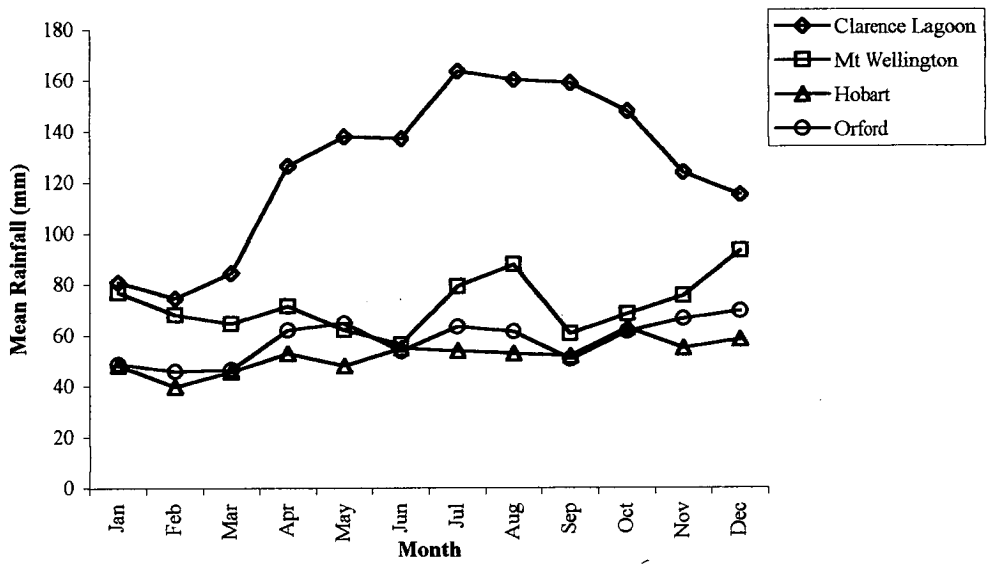

Figure 2.7 Mean monthly rainfall (mm) for the periods indicated in Table 2.1 for the Clarence Lagoon, Mt Wellington, Hobart and Orford areas.

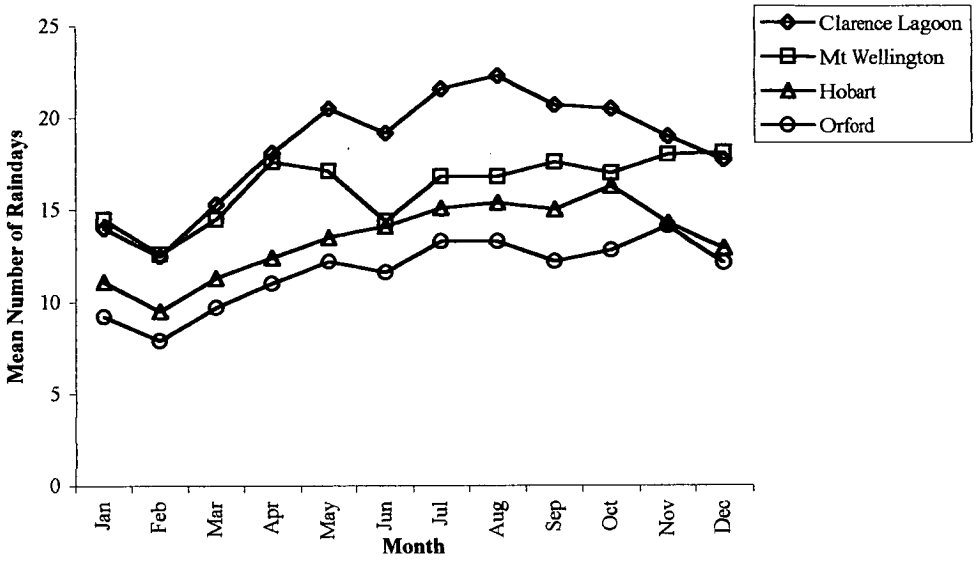

Figure 2.8 Mean number of raindays for the periods indicated in Table 2.1 for the Clarence Lagoon, Mt Wellington, Hobart and Orford areas.

Mean number of clear days was highest at Clarence Lagoon during summer and lowest at Hobart and Mt Wellington. Each area displays a distinctive pattern of clear days (Figure 2.9). Clear days decrease dramatically at Clarence Lagoon during winter, increase during autumn and winter at Orford, and generally decrease as the year progresses at both Hobart and Mt Wellington (due to the close geographical proximity of the two sites). Conversely, the number of cloudy days (Figure 2.10) is very similar between sites occurring at similar altitudes. Generally cloudy days are more frequent at high altitude and much more frequent at these sites during winter. At low altitude cloudy days tend to be equally frequent throughout the year, although at Orford, cloud cover increased slightly during summer months.

Cloud cover, however, has less effect on lizard activity at low altitude. Due to higher ambient air temperatures at lower altitude sites, lizards are often active under quite high cloud cover. At high altitude, substantial cloud often decreases air temperature enough to force lizards to seek refuge. Thus, while cloud is quite consistent between sites in summer, animals at low altitude are likely to be able to maintain greater levels of activity.

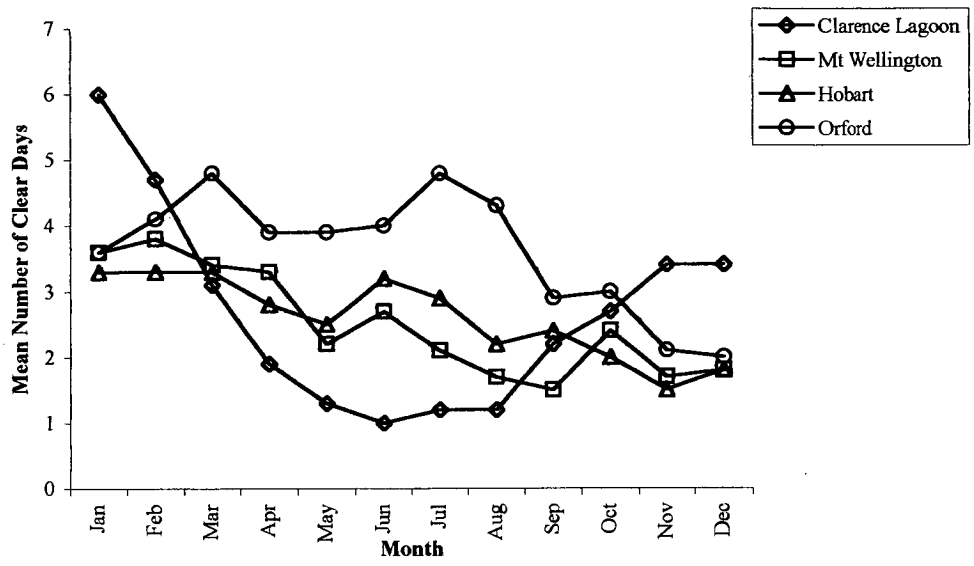

Figure 2.9 Mean number of clear days for the periods indicated in Table 2.1 for the Clarence Lagoon, Mt Wellington, Hobart and Orford areas.

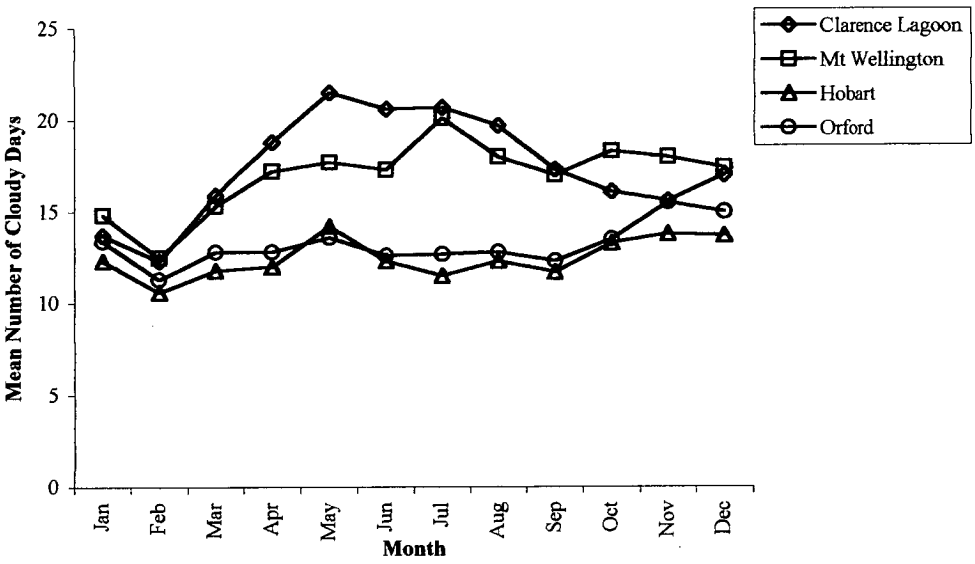

Figure 2.10 Mean number of cloudy days for the periods indicated in Table 2.1 for the Clarence Lagoon, Mt Wellington, Hobart and Orford areas.

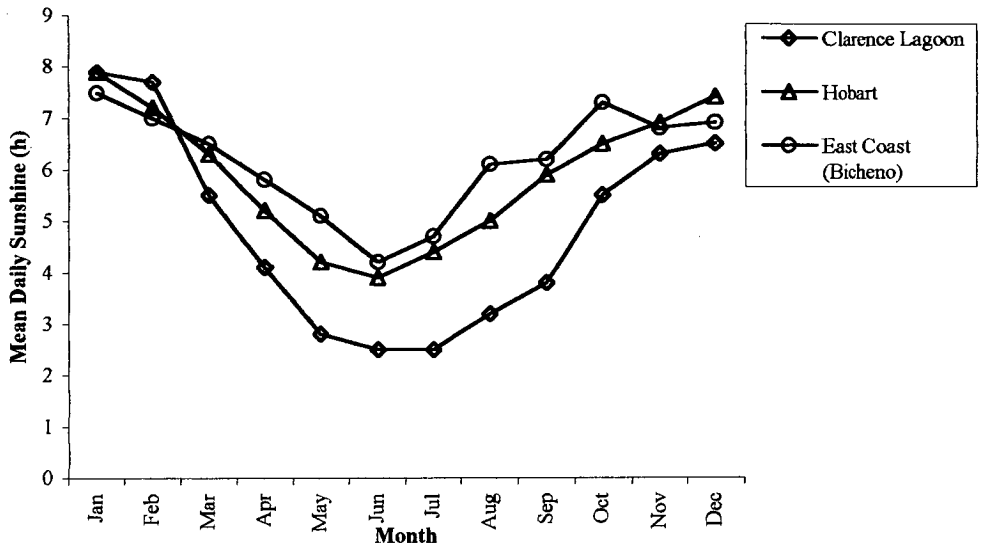

Figure 2.11 Mean daily sunshine (h) for the periods indicated in Table 2.1 for the Clarence Lagoon, Hobart and the East Coast (Bicheno). No data were available on daily sunshine for Orford or Mt Wellington. Bicheno (41° 52' S, 148° 18' E) represents the closest weather station to Orford at a similar longitude, elevation and aspect.

Mean daily sunshine (h) during summer is also quite consistent between sites (Figure 2.11). No data were available from the Mt Wellington and Orford stations; however, the proximity of Mt Wellington to Hobart must result in both sites having similar day lengths. The Orford site is also likely to be similar to the Bicheno station, which is also situated on the East Coast of Tasmania, at a similar longitude to the Orford site. Clearly data show that, during summer, daily sunshine is quite similar between all sites. During autumn and spring, it is somewhat decreased at the high altitude Clarence Lagoon site.

2.4 Lizard capture and housing

Lizards were captured at all sites by noose gun. This technique proved to be remarkably effective for capturing *N. metallicus* at high altitude; however, at lower altitude sites, significantly more effort was required due to the cryptic nature of animals from these populations (Chapter 4). At these sites noosing was supplemented with hand capture and fishing with a mealworm attached to cotton thread. Inactive lizards were also captured at

low altitude sites by turning rocks; however, this technique was very ineffective during summer when animals tended to retreat into clumps of grass and vegetation.

Following capture, animals were transported to the University of Tasmania reptile laboratory in cotton bags. Once in the laboratory, they were held under standard conditions (14L: 10D light cycle) with 10 hours of access to basking lamps. Overhead light was supplied by ultraviolet and normal fluorescent tubes (Grow-Lux) and three large phosphorus globes (2000 W each). Background temperatures were set at 12°C using an air conditioning unit. Animals were held in containers measuring 20 x 30 x 10 cm with netting placed over the top of the container to prevent escape. Light globes inside tin cans suspended over each box served as a basking source and provided a temperature gradient of 12 to 35°C. Each container was provided with a basking surface (terracotta pots) and ground cover (paper cat litter pellets). Water and food containers were also provided. Adult animals were fed three times a week with mealworms, cat food and mashed banana (one meal of each per week). Water (with added multivitamins) was provided *ad libitum.* Animals from which life history data were required were killed by placed them in a freezer at –20°C (Cogger, 1992). Further details of handling procedures are provided in subsequent chapters.

2.5 Permits

The research described was conducted under Ethics Permit 97046 issued by the University of Tasmania Animal Ethics Committee. Limited collection of reptiles in Tasmania occurred in National parks (permit number FA 97050). All collection of mainland animals was conducted under a permit issued by the Victorian Department of Conservation and Natural Resources. Importation of *N. metallicus* from Victoria was conducted under permit number 2855 issued by the Department of Environment and Land Management. Collection of animals from the Hobart area and Mt Wellington was conducted under express permission from relevant landowners and Hobart City Council.

CHAPTER THREE

Genetic Divergence between populations of *Niveoscincus metallicus*

3.1 Introduction

The allocation of specific or subspecific status to allopatric populations presumed to be closely related is one of the more perplexing problems facing systematists (Rose and Selcer, 1989; Desmore, Rose and Kain, 1992). Despite the prevalence of the biological species concept, which requires reproductive incompatibility, the majority of reptile species have been classified by morphological distinctiveness alone. Using this criterion the degree of differentiation required between allopatric populations is generally larger than that observed between sympatric species (Conant, 1963). The task of allocating specific or subspecific status between visually similar populations, which are geographically close, is therefore rarely attempted.

3.1.1 Genetic variability in *Niveoscincus metallicus*

There is considerable evidence that *Niveoscincus metallicus* displays some level of genetic variability across its range. The species is widespread in southeast Australia and Tasmania, ranging from sea level to alpine elevations (e.g. summit of Mt. Barrow at 1413 meters). It is also found in a variety of habitat types. Morphologically the species is quite variable; however, this variation can be observed both within and between populations on almost the same scale. The main exception to this generalisation is provided by the strongly striped and keeled specimens from southwest Tasmania, whose degree of differentiation and internal consistency, when compared to other populations, suggests some level of genetic distinction (M. Hutchinson, unpublished). Melville and Swain (1998) also indicated that there was a high level of intraspecific diversity in cytochrome b sequences between *N. metallicus* populations from central Tasmania and northeast Tasmania (Ben Lomond). This diversity was in the order of 12.2%.

3.1.2 Previous research on the genus *Niveoscincus*

There have been four previous studies examining phylogenetic relationships within *Niveoscincus*, the most recent of which probably provides the most reliable insight into the history of the group. In the earliest analysis Hutchinson *et al.* (1990) estimated that the group diverged recently, within the last 5 million years. They based this conclusion on the observation that fewer than 10 albumin ID units separated the Tasmanian endemic species. Hutchinson and Schwaner (1991), using allozyme electrophoresis, suggested that a single species probably gave rise to four modern lineages during the Pleistocene glaciations. Allozyme electrophoresis is a powerful method of examining gene flow between populations (Richardson *et al.*, 1986); however, use of electrophoretic distance data in phylogenetic studies is more controversial (Hutchinson and Schwaner, 1991). Hutchinson and Schwaner (1991) concluded that the inability of allozyme electrophoresis to resolve the phylogenetic relationships of *Niveoscincus* was a result of genetic divergence between the major lineages allowing homoplasy of allele mobility to affect data reliability.

Following these studies Melville and Swain (1998) examined the mitochondrial DNA cytochrome b region in an attempt to clarify the phylogeny of the group. They showed that the group was monophyletic with four distinct lineages. However, their phylogeny differed significantly from that of Hutchinson and Schwaner (1991). They tentatively proposed that the modern lineages of the group radiated from a heath dweller in the late Tertiary (2-7 million years ago). Subsequently Melville and Swain (2000b) examined the cytochrome oxidase (subunit I) region of the mitochondrial DNA fragment. Their findings again supported a late Tertiary evolution within the group; however, the topology created differed from that of their earlier study, and the phylogeny constructed better supported earlier theories of divergence patterns in *Niveoscincus* (Figure 3.1). They concluded that a ground dwelling species similar to *N. metallicus* probably gave rise to the group.

This thesis represents the only attempt to date to expand on current knowledge by examining genetic diversity within any of the presently accepted species. It examines evidence for possible vicariant biogeographic speciation within *N. metallicus*, including the role of past glacial events in shaping the present day distribution of the groups. To do this I use mitochondrial DNA (mtDNA) restriction analysis of the 12s-16s fragment

(2000bp's) along with sequencing procedures (16s, 583bp's) to test the null hypothesis that a single non-variable species is present. The results reported in this chapter of the thesis influenced field site selection in subsequent sections of the investigation. This was done to control for genetic differentiation between populations in later examinations of life-history and thermal biology.

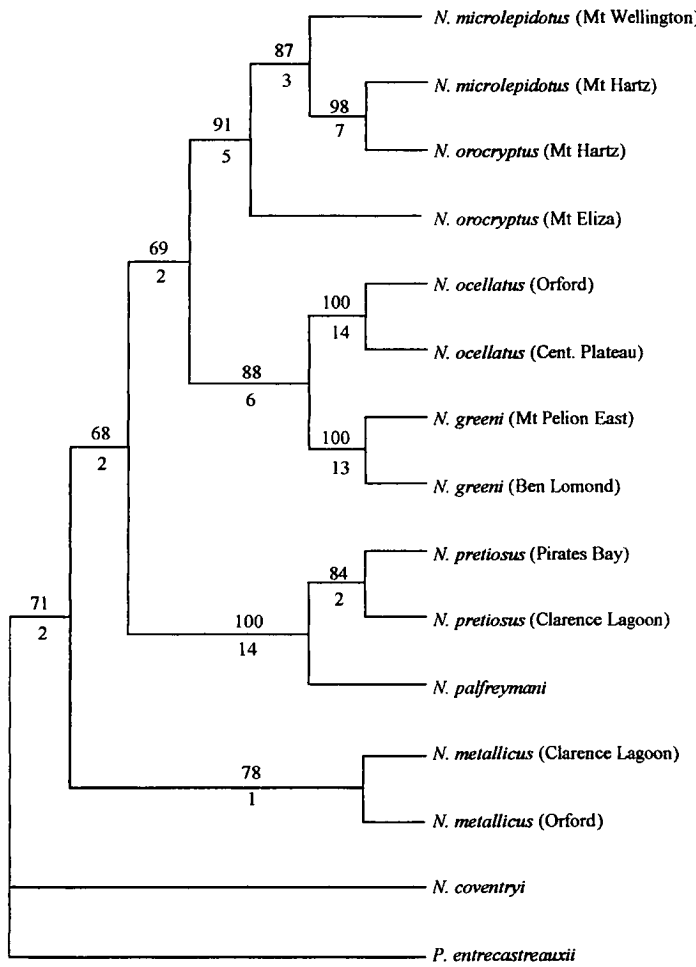

Figure 3.1 Parsimony bootstrap tree for *Niveoscincus* and the outgroup *Pseudemoia* from cytochrome oxidase (subunit 1) sequences. Bootstrap values (1000 replicates) for an unweighted analysis are provided above the lines. Decay indices are in bold type below the line. From Melville and Swain (1998).

3.1.3 Mitochondrial genes

The value of mitochondrial DNA as a tool for evolutionary investigations has been well established (Moritz *et al.*, 1987; Densmore, Rose and Kain, 1992). However, recently doubts have been raised about its reliability for predicting phylogenies (Hoelzer, 1997). Moore (1995) provided a clear discussion of the differences between gene trees and species trees. Species tree topology based on DNA-sequencing can be incorrectly inferred even though the gene tree has been correctly resolved (Wu, 1991; Doyle, 1992). This phenomenon may occur in situations where an ancestral species is polymorphic for the gene under examination and sorting of lineages is derived from the alternate haplotype (Moore, 1995; Hoelzer, 1997). The reliability of mt DNA is limited by lineage sorting, because the mitochondrial genes are inherited as a single linkage group, and therefore does not provide independent estimates of the species tree. Despite these objections, Moore (1995) concluded that a mitochondrial gene tree is more likely to reflect the species tree than its nuclear counterpart.

A further point which supports the above conclusion is that gene trees are more likely to track species trees if the ancestral species population size is small (Moore, 1995). The population genetics of the mitochondrial genome are determined by an effective population size that is one fourth the size of the nuclear genome. This occurs because mt DNA is haploid and only inherited maternally. However, Hoelzer (1997) suggested two scenarios that may result in the mitochondrial gene tree being a less reliable indicator of the species tree than nuclear DNA information. The two situations he examined, polygyny and female philopatry, both reduce the effective population size of nuclear genes relative to mitochondrial haplotypes. Moore (1997) agreed that both of these phenomena may result in the nuclear tree being the superior choice for phylogenetic investigations. However, he answered Hoelzer's rhetorical question "Is mtDNA a useful source of data for the estimation of species trees" by stating that there were " Very few circumstances where the mt-haplotype tree would not be the best bet as the true species tree". Moore (1995) provided a theoretical basis for preferring mt-DNA trees over nuclear-gene trees when examining phylogenies. His argument is based on a number of assumptions, including neutral nucleotide substitution, panmictic (randomly interbreeding) species populations and equal reproductive success for both males and females (Moore, 1997). The biology of *N. metallicus* meets these assumptions very well.

Sampling multiple unlinked genes would increase the probability of correctly inferring a species tree. However, as mt DNA genetic material is inherited as a single linkage group it provides only one independent estimate of a species tree. Despite this, it appears that a large number of nuclear-gene trees are required to improve upon the confidence based on the mitochondrial tree alone (Moore, 1995).

The mitochondrial genes encoding the 12s and to a less extent the 16s ribosomal RNA have been used in a number of phylogenetic investigations within reptiles and amphibians to examine a wide range of divergence times (Hedges *et al.* 1991; Thorpe *et al.* 1993; Thorpe *et al.* 1994; Heise *et al.* 1995; Caccone *et al.* 1997; Chippindale *et al.* 1998; Harris *et al.* 1998; Keogh, 1998; Keogh *et al.* 1998; Caccone, 1999; Georges *et al.*, 1999; Harris and Arnold, 1999; Honda *et al.* 1999; Parkinson, 1999). Mitochondrial ribosomal RNA coding genes generally evolve more slowly than their protein coding counterparts. Chippindale *et al.* (1998), in an examination of the phylogeny of *Abronia* (Anguidae: Gerrhonotinae), found that cytocrome *b* was roughly twice as variable as 12s sequences. Caccone *et al.* (1997) also found that cytochrome *b* genes evolve at nearly twice the rate of ribosomal genes in European newts (Genus *Euprocus*). However, they also indicated that cytocrome *b* rates were 3-7 times lower in salamanders than in other ectotherms. Generally, published evolutionary rates for mtDNA ribosomal genes in endotherms and ectotherms are 0.5 to 1% / million years for transitions plus transversions and 0.14% / million years for transversions only (Meyer and Wilson, 1990; Mindell and Honeycutt, 1990; Hillis and Dixon, 1991; Kraus and Miyamoto, 1991; Allard *et al.* 1992; Meyer, 1993; Ritchie *et al.* 1996). Despite the slower rate of change within mt DNA coding for ribosomal RNA, these segments still provide powerful tools for examining more recent divergences.

3.2 Materials and Methods

3.2.1 Sampling and site selection

I collected 140 specimens of *N. metallicus* from 13 localities, 11 from Tasmania and 2 from Victoria (Fig. 3.2). These sites broadly cover the geographic range of the species from south central Victoria to southern Tasmania, and also encompass the altitudinal extremes of the animal. Animals were transported to the University alive, where tail sections were removed and stored for subsequent DNA extraction at -80°C. I extracted DNA using Chelex techniques outlined in Walsh *et al.*, (1991).

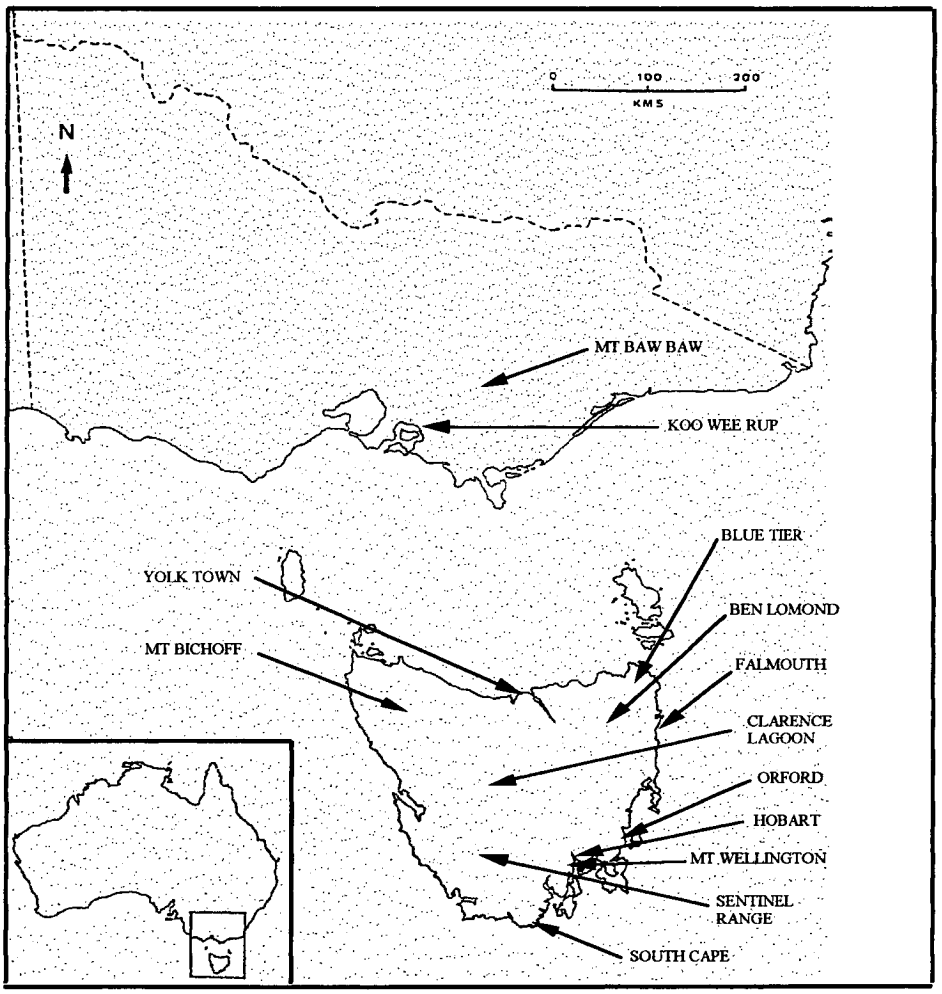

Figure 3.2 Collection sites for *Niveoscincus metallicus* from Tasmania (11 sites) and Victoria (2 sites).

3.2.2 Polymerase Chain Reaction (PCR)

Precautions were taken during the preparation of PCR reactions to minimise the risk of contamination from foreign DNA and consequent false positive amplifications. All PCR reactions were conducted in a room specifically dedicated to this purpose. Every set of PCR reactions was conducted with positive (both *N. metallicus* and Orange Roughy (*Hoplostethus atlanticus*)) and negative (no DNA) controls. The presence of any amplification in the negative control indicated the introduction of foreign DNA. Any amplification series in which this occurred was abandoned.

Synthetic oligonucleotide primers were used to amplify an approximately 2000-bp portion of the 12s to 16s fragment of the mitochondrial genome. Primers used were 16sbrH (5' CCG GTC TGA ACT CAG ATC ACG T 3') and 12SA-L (5' AAA CTG GGA TTA GAT ACC CCA CTA T 3') (Meyer *et al.*, 1990). Reactions contained 1.5 mM $MgCl_2$. DNA template was added to reactions in volumes ranging from 3 µl to 6 µl depending upon the extraction procedure employed. Thermal cycling was performed in a Corbett FTS-320 thermal cycler. All cycling methods began with a hot start at 94°C for 4 minutes. Double-stranded amplification involved 35 cycles of denaturation at 94°C (30 seconds), annealing at 55°C (30 seconds) and extension at 72°C (1 minutes). Cycling finished with a final extension time of 5 minutes at 72°C followed by a rapid ramp to a 4°C hold. This procedure was also tried on a MJ Research PTC-200 Peltier thermal cycler; however, slower ramp speeds in the Corbett cycler resulted in superior amplification of the large (2000-bp) DNA fragment. The effectiveness of PCR reactions was measured by gel electrophoresis and ethidium bromide staining. Fragments were visualised under ultraviolet light (302 nm).

3.2.3 Mitochondrial DNA Restriction fragment length polymorphism analyses (RFLP)

Six potentially useful restriction enzymes were used to digest the amplified PCR products of all animals collected from each site: Bsl I (5' CCNNNNN^NNGG 3'); BstU I (5' CG^CG 3'); Hae III (5' GG^CC 3'); HinP1 I (5' G^CGC 3'); Rsa I (5' GT^AC 3'); and Spe I (5' A^CTAGT 3').

All digestions of PCR products were performed in either 1.5 ml microcentrifuge tubes at 37°C in a water bath, or in 250 µl tubes at 55°C in the MJ Research PTC-200 Peltier thermal cycler, for 8 to 12 hours. Digestion reactions contained 10 µl DNA and specific amounts of enzyme, buffer and BSA as indicated through the place of perchase (New England Biolabs, NEB). Reactions were made up to 20 µl with distilled H_2O.

Digested PCR products were visualised by gel electrophoresis and ethidium bromide staining. A volume of restriction digest (10 µl) was loaded with 2 µl of Bresatec 2x loading buffer prior to electrophoresis on a 3% agarose gel in TBE buffer. After electrophoresis, gels were rinsed with water, and DNA fragments were visualized under ultraviolet light (302 nm). Profiles were recorded on photographs. Approximately 3 µl of Brestec DMW-100L 100-bp ladder was run concurrently with each group of restriction digests to determine mtDNA fragment size and to enable comparison of restriction profiles between gels.

A contingency χ^2 test on pooled restriction data was used to determine whether composite haplotype frequencies were heterogeneous across samples. Since all analyses were based on relatively small populations ($n<17$), the Monte Carlo randomisation approach of Roff and Bentzen (1989) was used as this eliminates the need to pool rare haplotypes. One thousand randomisations were used and the number of times each randomised replicate was greater than or equal to the observed value divided by 1000 provided an estimate of the probability of obtaining the result by chance alone. No further analyses to examine relationships between samples were conducted. This was because dendrograms examining relationships between groups were produced by sequencing the 16s region (see later) and relationships between groups were stark with no shared haplotypes between regions, and little or no variation within regions.

3.2.4 Nucleotide sequence divergence between site

A 584-bp region of the 16s rRNA mtDNA gene was sequenced for animals from each of the zones identified using restriction techniques. A total of 17 animals were sequenced for the 16s rRNA segments from the same 12s-16s rRNA section amplified in the previous section. Sites used for this section were Orford, Clarence lagoon, South Cape, Koo Wee

Rup, Ben Lomond, Falmouth, Mt Bishoff and one *Pseudemoia entrecasteauxii* specimen from SouthernTasmania selected as an outgroup specimen. Conditions and descriptions of PCR amplification are described in Section 3.2.2. PCR product was purified prior to nucleotide sequence determination using the Qiagen QIAquick PCR purification kit (50). Purified DNA was stored at 4°C.

The nucleotide sequence of PCR products was determined using automated sequencing procedures based on the dideoxy chain termination technique (Sanger *et al.*, 1977). All nucleotide sequences were resolved on an ABI PRISM-377 DNA Sequencer (Perkin Elmer).

Sequences were aligned using the Sequence Navigator application (Perkin Elmer) using the CLUSTAL option. Alignments were unambiguous despite the presence of a small insertion/deletion observed between the outgroup and the *N. metallicus* populations. Percent divergences were calculated and phylogenetic analyses performed using PAUP version 4.0. Two types of parsimony analysis were used to compare nucleotide sequences. These differed in the relative weighting given to the two types of nucleotide substitutions, transitions (TIs) and transversions (TVs). Weightings were varied because the probability of a base being replaced by another base varies with the base chemistry. There are four nucleotide bases represented in DNA: the purines, adenine and guanine; and the pyrimidines, thymine and cytosine. Brown *et al.* (1982) reported that the like replacement (TI) is more likely to occur than is a TV which requires a major change in chemical structure. Because TVs occur less frequently than TIs, they are less likely to be affected by the presence of homoplasious characters; i.e. those characters that are shared between species, but are not derived from a common ancestor. The STEPMATRIX option of PAUP was used to weight base substitutions as either unweighted or TRs as 3 and TIs as 1. This was based on the observed frequencies of these substitutions.

The HEURISTIC search-option within PAUP was used to perform weighted and unweighted parsimony. Both analyses were performed with the STEPWISE-ADDITION option in effect. When more than one most-parsimonious tree was obtained, the strict consensus tree (Rohlf, 1982) was constructed. This tree maintains only those discrete groupings that are present in all of the most-parsimonious trees obtained.

Confidence in the weighted strict consensus tree topology was assessed with the bootstrapping option (Felsenstein, 1985). This was done using similar heuristic search options on 1000 replicate data sets. Bootstrapping is now a common method for assessing phylogenetic analyses (Hillis and Bull, 1993). Under conditions of equal rates of change, symmetric phylogenies, and internodal changes of ≤20% of characters, bootstrap proportions of ≥70% usually correspond to a probability of ≥95% that the corresponding clade is real (Hillis and Bull, 1993). Consequently, in this study bootstrap values ≥70% were considered to indicate a high degree of confidence in the branching.

The neighbour-joining tree construction algorithm (Saitou and Nei, 1987) was used to analyse similarity estimates calculated from pair-wise comparisons of DNA sequences. The Kimura 2-parameter model of sequence evolution (Kimura, 1980) was used to correct calculations of pair-wise distance values from DNA sequences for multiple substitutions at nucleotide positions which could underestimate these distances. Analysis was conducted using PAUP (version 4.0). The neighbour-joining tree was constructed for unweighted character state changes only. Again bootstrap values were applied to assess confidence in groupings observed in consensus trees produced from the independent analysis of 1000 replicate data sets.

3.3 Results

3.3.1 Mitochondrial DNA Restriction fragment length polymorphism analyses (RFLP)

The composite haplotypes obtained for *N. metallicus* populations from Tasmania and Victoria are displayed in Table 3.1. Numbers of haplotypes observed at each site are also shown. Of the six restriction enzymes used in the analysis, four showed some level of variation. Therefore, haplotypes displayed in Table 3.1 show composite profiles for all four variable enzymes.

A visual distribution of these composite haplotypes is presented in Figure 3.3. Each individual restriction profile is displayed in Table 3.2. The 13 sites can be divided into 5 geographic regions. This differentiation is supported by the Monte Carlo test which produced a probability of obtaining the above haplotype distributions by chance alone of

less than 0.001. Thus I rejected the null hypothesis that there was no genetic variation across the geographic range of the animal.

Table 3.1 Distribution of composite mtDNA haplotypes of *Niveoscincus metallicus* (restriction profiles), representing the four restriction enzymes, *Rsa I, Spe I, Bsl I* and *Hae III* respectively, across all populations examined (n = number sampled).

Site	*AAAA*	*AAAB*	*ABAA*	*BABA*	*AACA*	*CCDA*	*AAEA*
Clarence Lagoon	15	1					
Orford	13	2	1				
Mt. Wellington	17						
Hobart	16						
Sentinel Range	2						
Ben Lomond	1						1
Falmouth							4
Blue Tier							4
Mt. Bishoff					16		
York Town					3		
Koo Wee Rup						16	
Mt. Baw Baw						14	
South Cape				16			

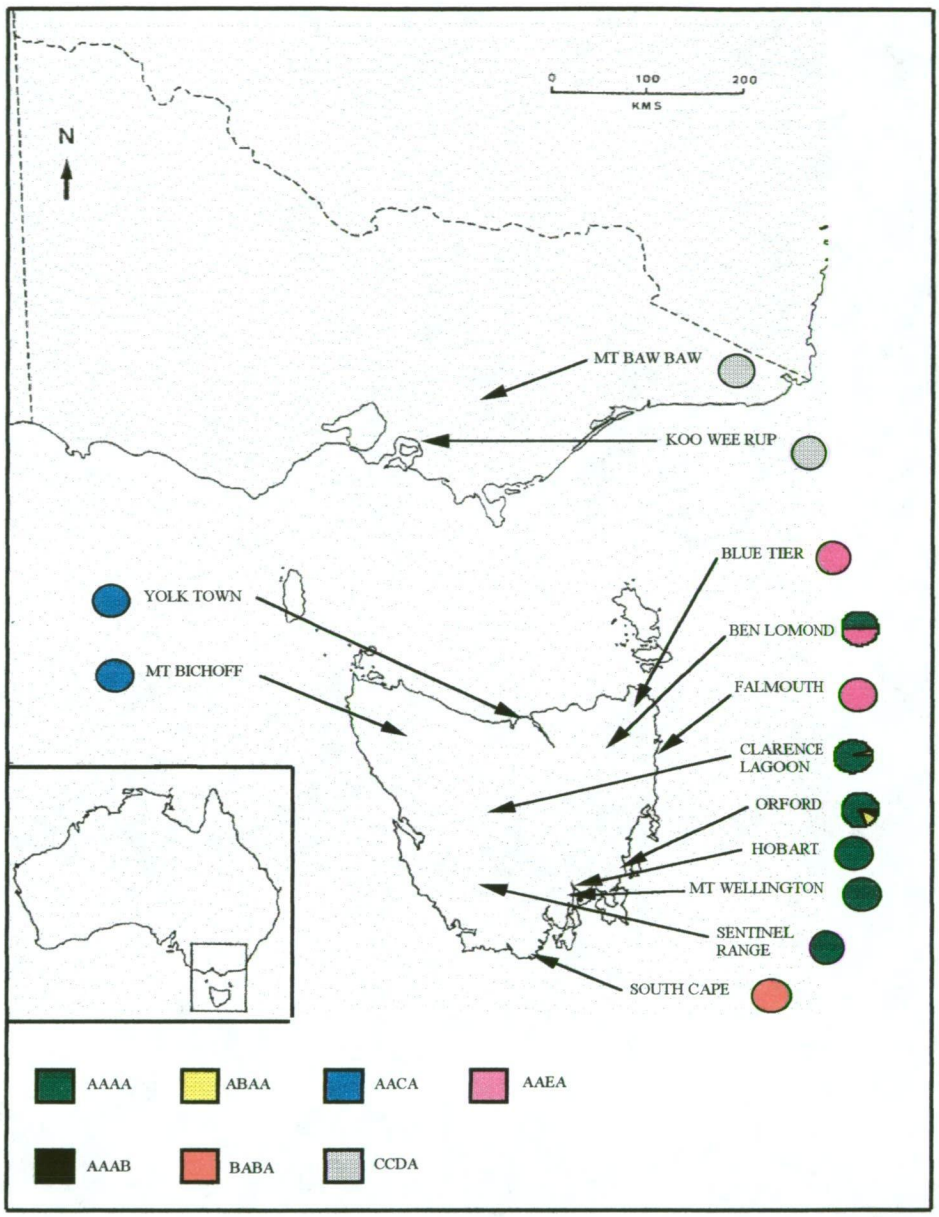

Fig 3.3 The distribution of composite haplotypes in *Niveoscincus metallicus* from Tasmania and Victoria. Haplotypes are designated by a four letters code representing the restriction profiles produced from the enzymes *Rsa I, Spe I, Bsl I* and *Hae III* respectively.

Animals from the Clarence Lagoon, Hobart, Mt. Wellington, Orford and Sentinel Range sites all form one group with the majority of animals displaying the composite haplotype AAAA. One animal from Ben Lomond also showed this pattern. The Orford and Clarence lagoon sites showed some internal diversity. Both of these sites showed the AAAB

haplotype and the Orford site also had one animal with a third ABAA haplotype. These patterns were not observed outside this group. All animals from Victoria displayed the CCDA haplotype, while within Tasmania, northeastern (AAEA), northwestern (AACA) and southern (BABA) composite haplotypes were also evident.

Table 3.2 Fragment sizes of each restriction profile produced from *Niveoscincus metallicus* by the four restriction enzymes, *Rsa I, Spe I, Bsl I* and *Hae III*. Values are given for fragments, which were resolved on 3% agarose gels.

	Restriction Profile				
Enzyme	**A**	**B**	**C**	**D**	**E**
Hae III	1000	-			
	-	800			
	495	495			
	380	380			
	-	200			
	100	100			
Rsa I	-	-	940		
	570	570	-		
	-	-	520		
	470	470	-		
	-	-	390		
	-	370	-		
	270	-	-		
	260	260	-		
	190	190	190		
	-	-	100		
Spe I	-	1260	-		
	-	-	800		
	740	740	740		
	680	-	-		
	580	-	-		
	-	-	430		
	-	-	170		
	-	-	120		

Bsl I	820	820	-	-	820
	690	-	690	-	-
	-	-	-	680	-
	-	-	610	-	-
	-	-	-	490	490
	-	480	-	-	-
	410	410	410	410	410
	-	-	-	210	-
	-	-	200	200	200
	-	170	-	-	-

Table 3.2 Cont

3.3.2 Nucleotide sequence divergence between sites

Sequence variation within a 584 bp portion of the 16s rRNA gene was observed at a total of only 33 nucleotide positions (Figure 3.4). Variation was informative at only 23 of these. The variation at the remaining 10 sites was observed in a single individual alone, and therefore was not interpreted as a shared derived character.

Phylogenetic analyses of protein coding genes, such as the cytochrome *b* gene of mtDNA, must consider the codon position at which nucleotide variation occurs. This is a result of changes in amino acid sequences caused by nucleotide substitutions at either the first or second codon position. The position of nucleotide substitutions is not considered in this analysis as the 16s rRNA gene is not a protein-coding region and therefore nucleotide changes in this gene do not influence amino acid production.

In all but one case sequences were obtained from more than one individual per site, and in all cases multiple animals were sequenced per region (identified from RFLP analysis). Levels of variation (measured as Kimora 2-parameter estimates, Table 3.3) within sites were in most cases low. Animals from the Mt Bichoff site showed no intrasite variation at all, while animals from Victoria showed less than 0.18% variation. South Cape animals also showed high levels of internal consistency with a maximum percentage variation of 0.35%. Levels of differentiation were higher within the northeastern region, with animals from Falmouth showing 0.53% variation and the Ben Lomond individual displaying a maximum of 1.3% divergence from Falmouth lizards.

Orford 1	AGCTGTTGCA	CCGTTTGGGG	GTCCTGATCC	AACATCGAGG	TCGTAAACCT	TCTTGTCGAT
Orford 2	----------	----------	----------	----------	----------	----------
Orford 3	----------	----------	----------	------N---	----------	----------
Clarence Lag 1	----------	---------A	----------	----------	----------	----------
Clarence Lag 2	----------	----------	----------	----------	----------	----------
Southcape 1	----------	----------	----------	----------	----------	----------
Southcape 2	----------	----------	----------	----------	----------	----------
Southcape 3	----------	----------	----------	----------	----------	----------
Koo Wee Rup 1	----------	----------	----------	-----N----	----------	----------
Koo Wee Rup 2	----------	----------	----------	----------	----------	----------
Koo Wee Rup 3	----------	----------	----------	-----N----	----------	----------
Mt Bichoff 1	----------	----------	----------	----------	----------	----------
Mt Bichoff 2	----------	----------	----------	----------	----------	----------
Ben Lomond 1	----------	----------	----------	----------	----------	----------
Falmouth 1	G------T--	----------	----------	------A---	----------	----------
Falmouth 2	G------T--	----------	----------	----------	----------	----------

Orford 1	AGGGACTCTT	GAAGAAGATA	GCGCTGTTAT	CCCTGGGGTA	ACTTGGTTCG	TTGTTCAGTA
Orford 2	----------	----------	----------	----------	----------	----------
Orford 3	----T-----	----------	----------	----------	----------	-------A--
Clarence Lag 1	----------	----------	----------	----------	----------	----------
Clarence Lag 2	----------	----------	----------	----------	----------	----------
Southcape 1	----------	----------	----------	----------	----------	---------G
Southcape 2	----------	----------	----------	----------	----------	----------
Southcape 3	----------	----------	----------	----------	----------	---------G
Koo Wee Rup 1	----------	----------	----------	----------	----------	--------A-
Koo Wee Rup 2	----------	----------	----------	----------	----------	--------A-
Koo Wee Rup 3	----------	----------	----------	----------	----------	--------A-
Mt Bichoff 1	-T--------	----------	----------	----------	----------	----------
Mt Bichoff 2	-T--------	----------	----------	----------	----------	----------
Ben Lomond 1	-T--------	----------	----------	----------	----------	--------A-
Falmouth 1	----------	----------	----------	----------	----------	--------A-
Falmouth 2	----------	----------	----------	----------	----------	--------A-

Figure 3.4 Partial 16S rRNA nucleotide base sequence obtained from 16 *Niveoscincus metallicus* from 7 sites around Tasmania and Victoria. The sequences are represented in a 5' to 3' direction. Dashes indicate sequence identity with reference to initial sequence. Asterisks' represent insertions or deletions.

Orford 1	AGACTGGGTC	GG**TTTATT	CATTTGACTT	GTTGGTCTGG	TTGAGAGGGG	TTGGCTCTGT
Orford 2	----------	----------	----------	----------	----------	----------
Orford 3	----------	----------	----------	----------	----------	----------
Clarence Lag 1	----------	----------	----------	----------	----------	----------
Clarence Lag 2	----------	----------	----------	----------	----------	----------
Southcape 1	----------	----C-----	----------	--------A-	----------	----------
Southcape 2	----------	----C-----	----------	--------A-	----------	----------
Southcape 3	----------	----C-----	----------	--------A-	----------	----------
Koo Wee Rup 1	----------	----C-----	----------	--------A-	A---------	----------
Koo Wee Rup 2	----------	----C-----	----------	--------A-	A---------	----------
Koo Wee Rup 3	----------	----C-----	----------	--------A-	A---------	----------
Mt Bichoff 1	----------	----C-----	----------	--------AA	-------T--	-A--------
Mt Bichoff 2	----------	----C-----	----------	--------AA	-------T--	-A--------
Ben Lomond 1	----------	----------	----------	--------A-	A---------	----------
Falmouth 1	----------	----------	----------	--------A-	A---------	----------
Falmouth 2	----------	----------	----------	--------A-	A---------	----------

Orford 1	GCTCGGAAGT	TTTGCTTTGT	TCCGAAGTCG	CCCCAACTTA	AAACTTGTGC	TATTTCGTGG
Orford 2	----------	----------	----------	----------	----------	----------
Orford 3	----------	----------	----------	----------	----------	----------
Clarence Lag 1	----------	----T-----	----------	----------	----------	----------
Clarence Lag 2	----------	----T-----	----------	----------	----------	----------
Southcape 1	----------	----------	----------	----------	--------A-	----------
Southcape 2	----------	----------	----------	----------	--------A-	----------
Southcape 3	----------	----------	----------	----------	--------A-	----------
Koo Wee Rup 1	----------	----------	----------	----------	----------	----------
Koo Wee Rup 2	----------	----------	----------	----------	----------	----------
Koo Wee Rup 3	----------	----------	----------	----------	----------	----------
Mt Bichoff 1	----------	----------	----------	----------	----------	-------G--
Mt Bichoff 2	----------	----------	----------	----------	----------	-------G--
Ben Lomond 1	----------	----------	----------	----------	----------	----------
Falmouth 1	----------	----------	----------	----------	----------	----------
Falmouth 2	----------	----------	----------	----------	----------	----------

Figure 3.4 Continued.

Orford 1	TGATA*TAGT	GTTAGTTTTA	AGCTCCACAG	GGTCTTCTCG	TCTTATGTGT	TTATTCAAGC
Orford 2	----------	----------	----------	----------	----------	----------
Orford 3	----------	----------	----------	----------	----------	----------
Clarence Lag 1	----------	----------	----------	----------	----------	----------
Clarence Lag 2	----------	----------	----------	----------	----------	----------
Southcape 1	----------	----------	----------	----------	----------	----------
Southcape 2	----------	----------	----------	----------	----------	----------
Southcape 3	----------	----------	----------	----------	----------	----------
Koo Wee Rup 1	--G-------	----------	----------	----------	----------	----------
Koo Wee Rup 2	--G-------	----------	----------	----------	----------	----------
Koo Wee Rup 3	--G-------	----------	----------	----------	----------	----------
Mt Bichoff 1	----------	----------	----------	----------	----------	----------
Mt Bichoff 2	----------	----------	----------	----------	----------	----------
Ben Lomond 1	----------	----------	----------	----------	----------	----------
Falmouth 1	----------	----------	----------	----------	----------	----------
Falmouth 2	----------	----------	----------	----------	----------	----------

Orford 1	TTTTGTACTT	GAAGATCAGT	TTTACTGGTT	GATTATAAGA	GACAGGTCCG	TTCTCATTTA
Orford 2	----------	----------	----------	----------	----------	----------
Orford 3	----------	----------	----------	----------	----------	----------
Clarence Lag 1	----------	----------	----------	----------	----------	----------
Clarence Lag 2	----------	---------N	----------	----------	----------	----------
Southcape 1	----------	--------AG	----------	----------	----------	----------
Southcape 2	----------	--------AG	----------	----------	----------	----------
Southcape 3	----------	--------AG	----------	----------	----------	----------
Koo Wee Rup 1	----------	----------	----------	----------	----------	----------
Koo Wee Rup 2	----------	----------	----------	----------	----------	----------
Koo Wee Rup 3	----------	----------	----------	----------	----------	----------
Mt Bichoff 1	----------	----------	----------	----------	----------	----------
Mt Bichoff 2	----------	----------	----------	----------	----------	----------
Ben Lomond 1	----------	----------	----------	----------	----------	----------
Falmouth 1	----------	----------	----------	----------	----------	G---------
Falmouth 2	----------	----------	----------	----------	----------	G---------

Figure 3.4 Continued.

Orford 1	GCCTTTCATA	CAAGTCTTTA	TTTAAAAGAC	AAGTGATTAC	GCTACCTTTG	CACGGTTAGG
Orford 2	----------	----------	----------	----------	----------	----------
Orford 3	----------	----------	----------	----------	----------	----------
Clarence Lag 1	----------	----------	----------	----------	----------	----------
Clarence Lag 2	----------	----------	----------	----------	----------	----------
Southcape 1	----------	----------	----------	----------	----------	----------
Southcape 2	----------	----------	----------	----------	----------	----------
Southcape 3	----------	----------	----------	----------	----------	----------
Koo Wee Rup 1	----------	----------	----------	----------	----------	----------
Koo Wee Rup 2	----------	----------	----------	----------	----------	----------
Koo Wee Rup 3	----------	----------	----------	----------	----------	----------
Mt Bichoff 1	----------	----------	----------	----------	----------	----------
Mt Bichoff 2	----------	----------	----------	----------	----------	----------
Ben Lomond 1	----------	----------	----------	----------	----------	----------
Falmouth 1	----------	----------	----------	----------	----------	----------
Falmouth 2	----------	----------	----------	----------	----------	----------

Orford 1	ATACCGCGGC	CGTTTAAAAT	GCTTCACTGG	GCAGGCAGCA	CCTTTAATAC	TTGTTTGGCT
Orford 2	----------	----------	----------	----------	----------	----------
Orford 3	----------	----------	----------	----------	----------	----------
Clarence Lag 1	----------	----------	----------	----------	----------	----------
Clarence Lag 2	----------	----------	----------	----------	----------	----------
Southcape 1	----------	----------	----------	----------	----------	----------
Southcape 2	----------	----------	----------	----------	----------	----------
Southcape 3	----------	----------	----------	----------	----------	----------
Koo Wee Rup 1	----------	----------	----------	----------	----------	----------
Koo Wee Rup 2	----------	----------	----------	----------	----------	----------
Koo Wee Rup 3	----------	----------	----------	----------	----------	----------
Mt Bichoff 1	----------	----------	-T--------	----------	----------	----------
Mt Bichoff 2	----------	----------	-T--------	----------	----------	----------
Ben Lomond 1	----------	----------	----------	----------	----------	------A---
Falmouth 1	----------	----------	----------	---A------	----------	------A---
Falmouth 2	----------	----------	----------	----------	----------	----------

Figure 3.4 Continued.

Orford 1	AAAGGCTGTG	TTTTTGGTAA	ACAGTTGGGA	CGG**GTTTG	CTGAGTTCCT	TATATAATTT
Orford 2	----------	----------	--------A-	----------	----------	----------
Orford 3	----------	----------	--------A-	----------	----------	----------
Clarence Lag 1	----------	----------	--------A-	----------	----------	----------
Clarence Lag 2	----------	----------	--------A-	----------	----------	----------
Southcape 1	----------	----------	--------A-	----------	----------	----------
Southcape 2	----------	----------	--------A-	----------	---------C	----------
Southcape 3	----------	----------	--------A-	----------	----------	----------
Koo Wee Rup 1	----------	----------	--------A-	---G------	----------	----------
Koo Wee Rup 2	----------	----------	--------A-	---G------	----------	----------
Koo Wee Rup 3	----------	----------	--------A-	---C------	----------	----------
Mt Bichoff 1	----------	----------	--------A-	----------	----------	----------
Mt Bichoff 2	----------	----------	--------A-	----------	----------	----------
Ben Lomond 1	----------	----------	-------AA-	----------	----------	----------
Falmouth 1	----------	----------	--------A-	----------	----------	----------
Falmouth 2	----------	----------	--------A-	----------	----------	----------

Orford 1	TAAACCTTCT	TTGTGGCACT	CCAGTGTCGG	TTGACAGTTT	AATG
Orford 2	----------	----------	----------	----------	----
Orford 3	----------	----------	----------	----------	----
Clarence Lag 1	----------	----------	----------	----------	----
Clarence Lag 2	----------	----------	----------	----------	----
Southcape 1	----------	----------	---A------	----------	----
Southcape 2	----------	----------	---A------	----------	----
Southcape 3	----------	----------	---A------	----------	----
Koo Wee Rup 1	----------	----------	----------	----------	----
Koo Wee Rup 2	----------	----------	----------	----------	----
Koo Wee Rup 3	----------	----------	----------	----------	----
Mt Bichoff 1	----------	----------	----------	----------	-*--
Mt Bichoff 2	----------	----------	----------	----------	-*--
Ben Lomond 1	----------	----------	----------	----------	----
Falmouth 1	----------	----------	----------	----------	--G-
Falmouth 2	----------	----------	----------	----------	----

Figure 3.4 Continued

Table 3.3 Sequence divergences, Kimura 2-parameter (below diagonal) and observed number of substitutions (above diagonal) for each pair-wise comparison of animals. Values in red are within site comparisons.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Orford 1	-	1	3	3	2	8	8	8	6	6	6	9	9	9	11	8	61
2. Orford 2	0.17	-	2	2	1	7	7	7	5	5	5	8	8	8	10	7	62
3. Orford 3	0.52	0.35	-	4	3	9	9	9	7	7	7	10	10	10	11	9	64
4. Clarence Lagoon 1	0.52	0.35	0.70	-	1	9	9	9	7	7	7	10	10	10	12	9	61
5. Clarence Lagoon 2	0.34	0.17	0.52	0.17	-	7	7	7	6	6	6	9	9	9	11	8	61
6. Southcape 1	1.40	1.22	1.58	1.58	1.22	-	2	0	8	8	8	11	11	13	15	12	68
7. Southcape 2	1.40	1.22	1.58	1.58	1.22	0.35	-	2	8	8	8	11	11	13	15	12	68
8. Southcape 3	1.40	1.22	1.58	1.58	1.22	0.00	0.35	-	8	8	8	11	11	13	15	12	66
9. Koo Wee Rup 1	1.05	0.87	1.22	1.22	1.04	1.40	1.40	1.40	-	0	1	9	9	7	8	6	63
10. Koo Wee Rup 2	1.04	0.87	1.22	1.22	1.04	1.40	1.40	1.40	0.00	-	1	9	9	7	9	6	63
11. Kuu Wee Rup 3	1.05	0.87	1.22	1.22	1.05	1.40	1.39	1.40	0.17	0.17	-	9	9	7	8	6	64
12. Mt Bichoff 1	1.57	1.39	1.75	1.75	1.57	1.93	1.93	1.93	1.58	1.58	1.57	-	0	12	16	13	64
13. Mt Bichoff 2	1.57	1.40	1.75	1.75	1.58	1.93	1.93	1.93	1.58	1.58	1.58	0.00	-	12	16	13	64
14. Ben Lomond	1.57	1.39	1.75	1.75	1.57	2.28	2.28	2.28	1.22	1.22	1.22	2.11	2.11	-	8	5	68
15. Falmouth 1	1.93	1.75	1.94	2.11	1.93	2.65	2.65	2.65	1.41	1.58	1.40	2.83	2.83	1.40	-	3	68
16. Falmouth 2	1.40	1.22	1.57	1.57	1.40	2.10	2.10	2.10	1.05	1.05	1.05	2.28	2.29	0.87	0.52	-	65
17. *P. entrecasteauxii*	11.5	11.7	12.1	11.5	11.5	12.5	12.9	12.5	11.9	11.9	12.1	12.1	12.1	12.9	13.0	12.3	-

Animals from central Tasmania (Clarence Lagoon and Orford) showed intra-regional variation levels ranging from 0.18% to 0.7%.

Inter-regional variation within the species was somewhat more pronounced with sequence divergences ranging from 0.87% to 2.83%. Animals from central Tasmania and Victoria showed slightly more than 1% divergence as did those from northeastern Tasmanian and Victoria. Animals from northwestern Tasmania were the most distinct genetically, and displayed their greatest differentiation from northeastern Tasmanian animals.

Unweighted parsimony analysis produced 15 equally parsimonious trees. A strict consensus of these 15 trees showed three distinct groupings, these being South Cape animals, Victorian and northeastern Tasmanian animals, and northwestern Tasmanian animals (Figure 3.5). The analysis was, however, unable to group central Tasmanian animals and shed no light on the relationships between groups due to the unresolved relationship between terminal groups.

Weighting (3:1) resulted in a reduction to only 3 equally parsimonious trees. A strict consensus of these trees is shown in Figure 3.6. The tree produced the same groups as the unweighted analysis; however, it was also able to place the central Tasmanian animals into a single clade. The topology produced from this analysis also separates the northwestern Tasmanian (Mt. Bichoff) group from other populations. Bootstrapping of the weighted consensus tree showed strong support for the northwestern Tasmanian (99), northeastern Tasmanian and Victorian (71), and southern Tasmanian (98) clades (Figure 3.7). Despite this, there was little support for the central Tasmanian clade (56). There was also little support for the topology of the tree towards its base, with bootstrap values less than 70. The analysis indicates that northwestern Tasmanian animals form a sister group to other populations. Following this split there is a secondary splitting of northeastern Tasmanian / Victorian and central Tasmanian / southern Tasmanian animals. There is, however, little support for this splitting (47) or for the subsequent separation of South Cape and central Tasmanian animals (13).

The topology of the neighbour-joining tree (Figure 3.8) is very similar to that of the weighted parsimony tree. The main change is the switching of South Cape animals from the central Tasmanian clade to the northeastern/Victorian clade; however, the

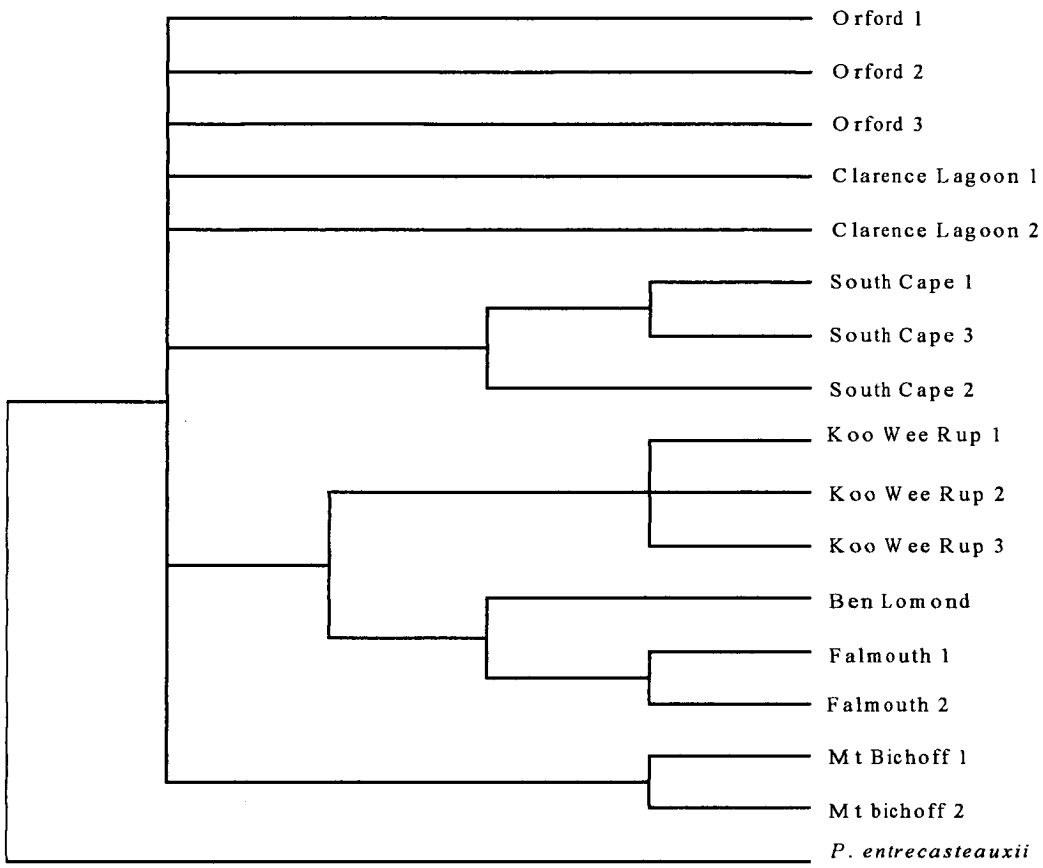

Figure 3.5 Unweighted strict consensus of fifteen equally parsimonious trees based on 584 bp of 16S rRNA sequence. The analysis was rooted with the outgroup *Pseudemoia entrecasteauxii*.

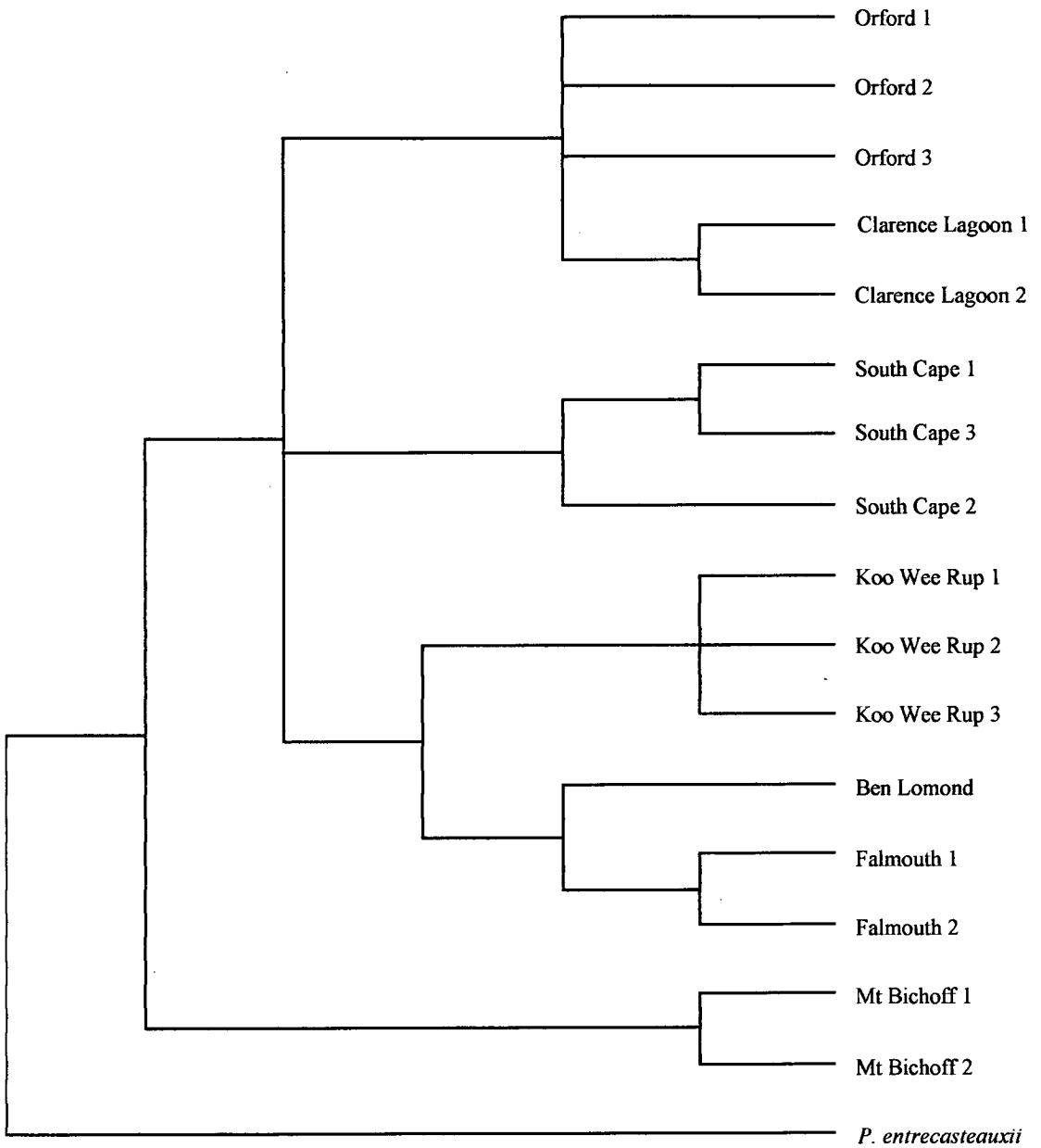

Figure 3.6 Weighted strict consensus of three equally parsimonious trees based on 584 bp of 16S rRNA sequence. Transversions:transitions weighted 3:1. The analysis was rooted with the outgroup *Pseudemoia entrecasteauxii*.

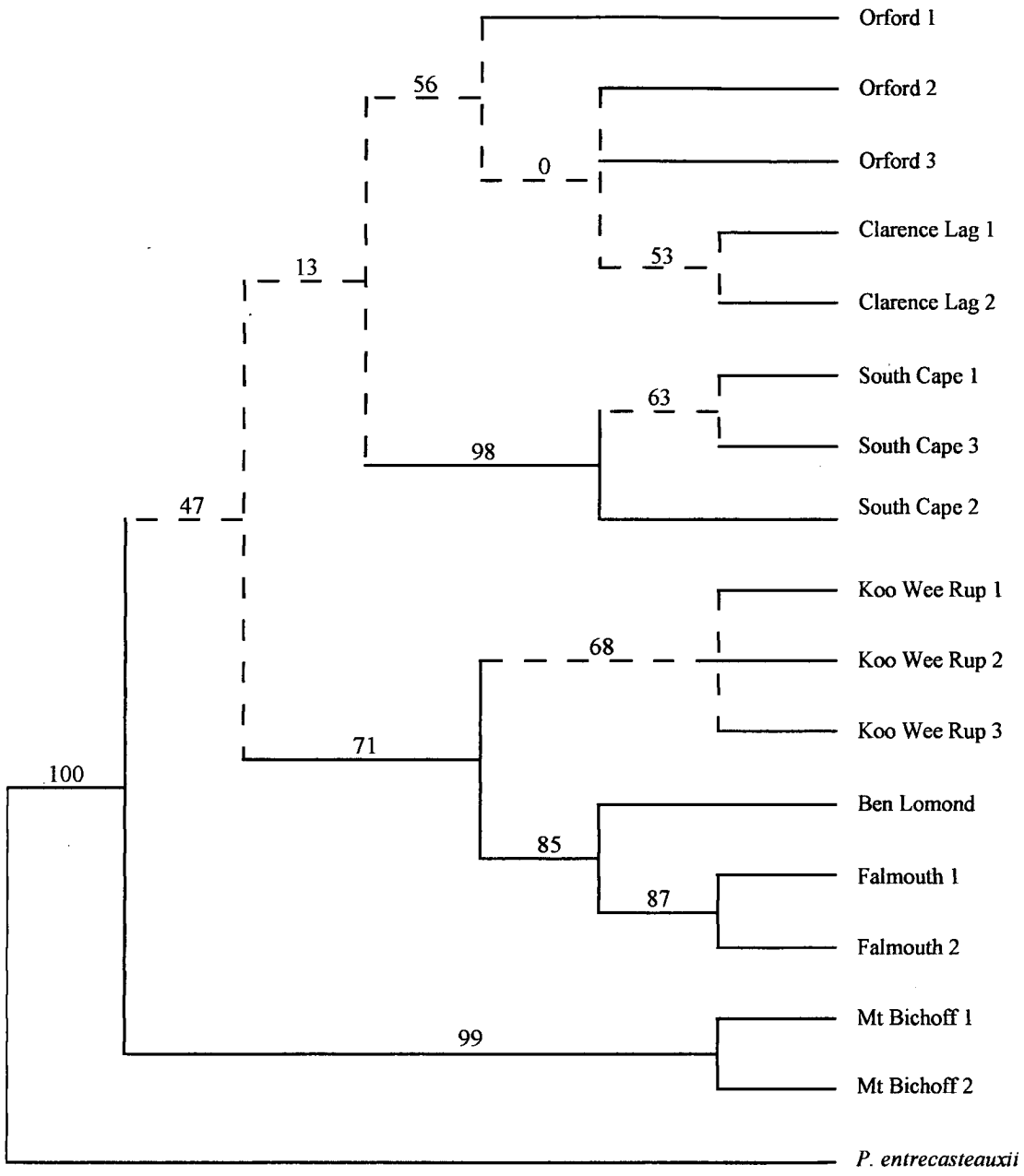

Figure 3.7 Parsimony bootstrap tree based on 584 bp of 16S rRNA sequence. The analysis includes the outgroup *Pseudemoia entrecasteauxii*. TV:TI weight of 3:1. Values at branch points indicate bootstrap values (1000 replicates) with estimates less than 70% shown by dashed lines.

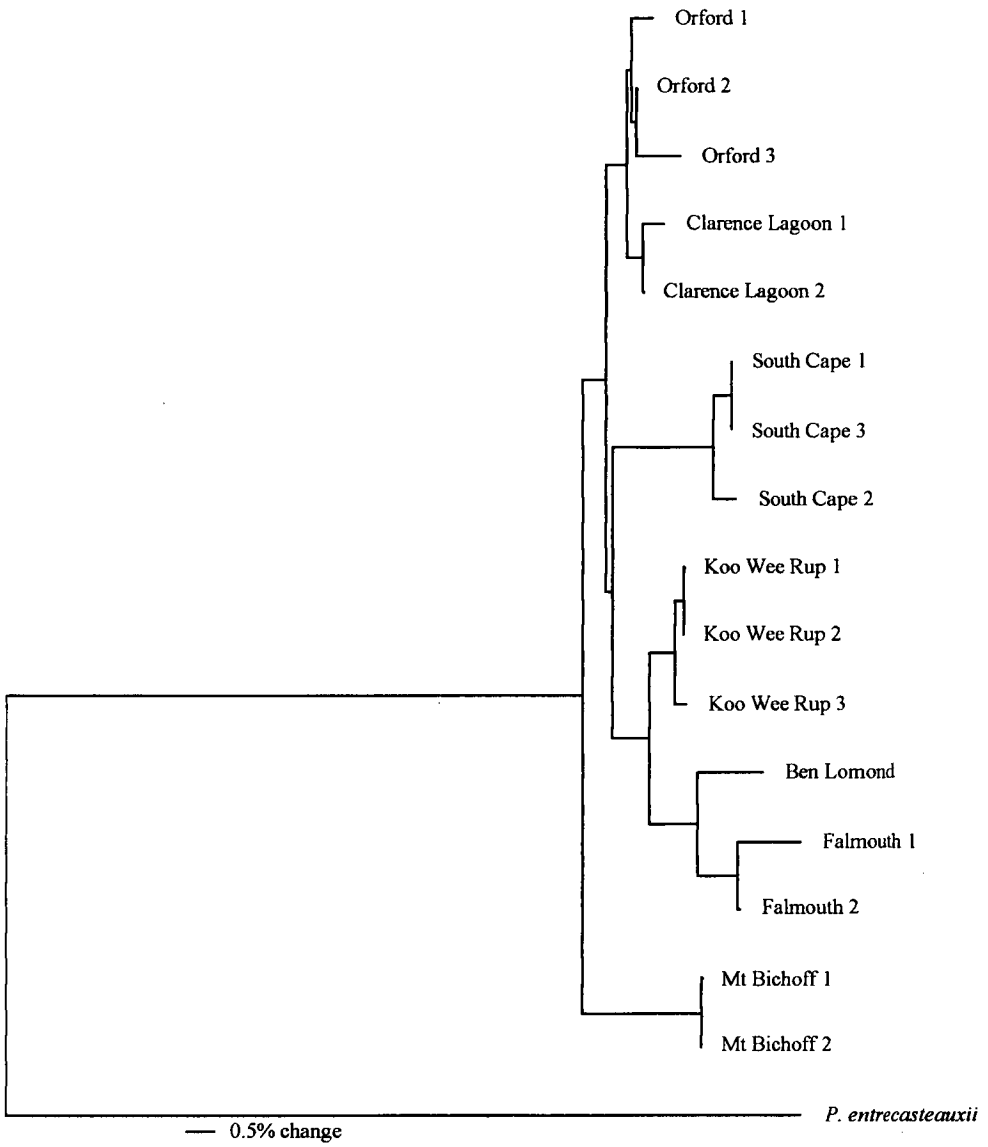

Figure 3.8 Neighbour-joining tree on 584 bp of 16s rRNA sequence. The analysis was rooted with the outgroup *Pseudemoia entrecasteauxii*. Pair-wise distances were calculated with the Kimura 2-parameter model of sequence evolution (Kimura 1980). Branch lengths are proportional to sequence divergence. The analysis was rooted using the outgroup *Pseudemoia entrecasteauxii*.

differentiation between groups in this section of the cladogram is very small, as is indicated by the short branch lengths. This makes resolution between these groups difficult. Bootstrapping the neighbour-joining tree resulted in an obvious change in its topology (Figure 3.9). This can happen when distances between groups are small, as is the case in this analysis. The main difference between this tree and Figure 3.8 is the movement of the Mt. Bichoff clade to within the main species group. However, bootstrap values across the entire base of this tree are less than 70 and so are unreliable. There is indeed very little support for any higher level groupings in this tree. Again, however, there is strong support for the existence of individual subgroups. The northeastern Tasmanian clade is supported by a bootstrap value of 86%, the Victorian clade is supported by a value of 73%, the Mt Bichoff clade is supported by a value of 100%, and the South Cape clade is supported by a bootstrap value of 99%. The central Tasmanian clade is again, however, poorly supported (35%).

3.4 Discussion

Both the restriction analysis and the 16S rRNA sequence evidence presented clearly reject the notion that *N. metallicus* is a single non-variable biological entity. *Niveoscincus metallicus* to date has been considered as one species, occupying a broad geographic and altitudinal range. The data presented here show clearly that there are in fact a minimum of four, and probably five, distinct groups occurring across the range examined. The mtDNA sequences of *N. metallicus* exhibited clear geographic structuring. Addressing this type of apparent divergence may shed light on events that can lead to speciation (Lawson *et al.*, 1991).

The main groups of sites, from this analysis, fall into the central Tasmanian (Type 1) cluster. The existence of this cluster is not well supported in any of the cladistic analyses; however, the RFLP analysis indicated that while some variation is evident within this grouping, all haplotypes observed were unique. A second well-supported grouping (Type 2) was observed in animals from only one site, this being South Cape. A northwest Tasmanian cluster (Type 3) was also well supported in all analyses. RFLP analysis showed clearly that two other groupings were evident, these being Northeast Tasmanian (Type 4) and Victorian (Type 5) clusters. However, all sequencing analyses indicated strong support for the grouping of these two clusters into one monophyletic clade, since a

high number of informative nucleotide base sequences were shared between them. The RFLP analysis also indicated that both Type 1 and Type 4 animals were present at the Ben Lomond site, suggesting that this site may represent a region of genetic exchange or flow between the two types following range expansion.

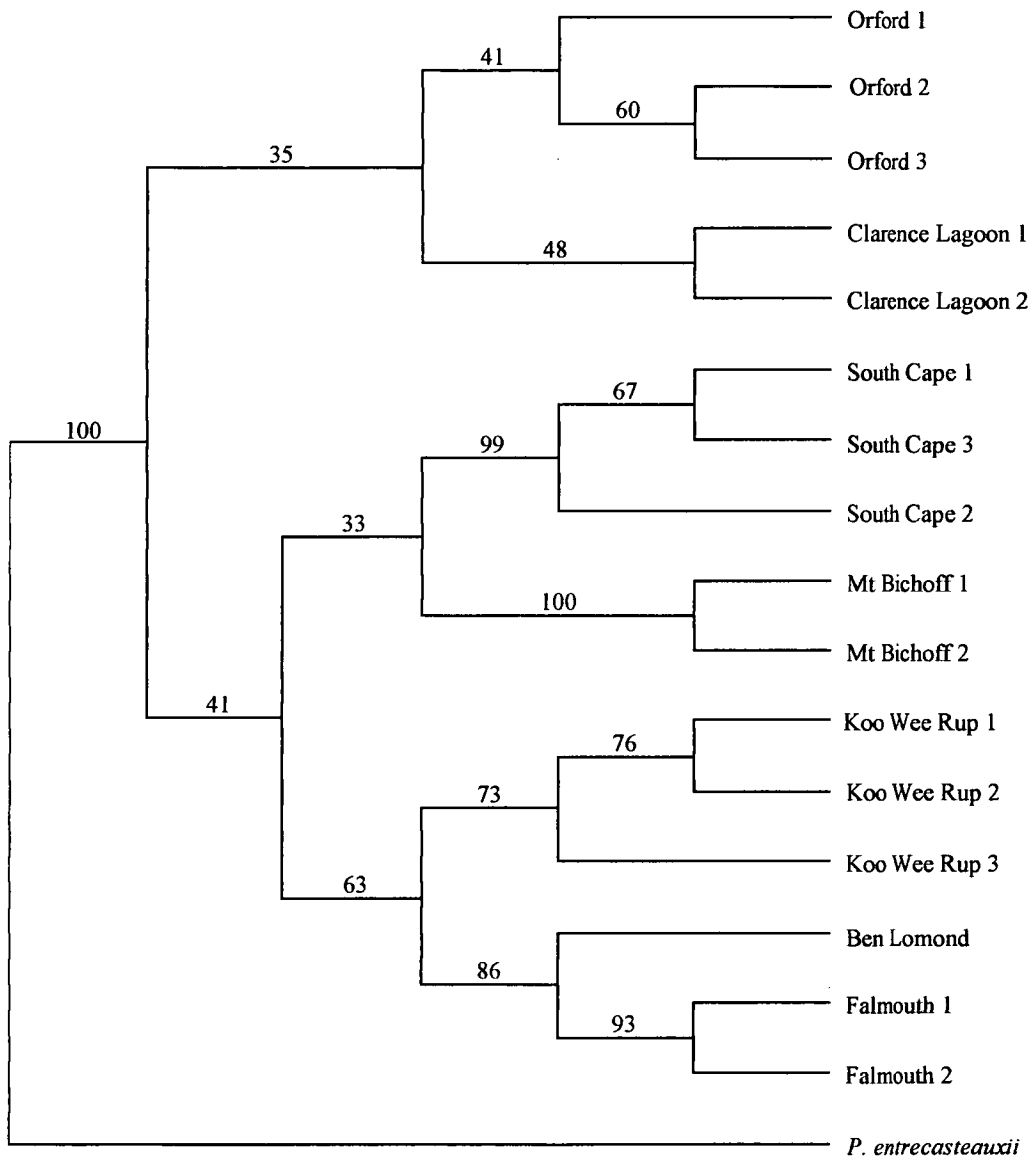

Figure 3.9 Neighbour-joining phenogram based on pair-wise distances calculated using the Kimura 2-parameter model of sequence evolution (Kimura, 1980). Values at each node represent bootstrap percentages derived from 1000 replicates. The analysis is rooted using the outgroup *Pseudemoia entrecasteauxii*.

The bootstrap values on the most parsimonious phylogeny of *N. metallicus* indicated a high level of support for three of the four clades, However, the bootstrap values towards the base of the tree were well below the 70% required to indicate significant support for a divergence. This lack of support is also evident in the neighbour-joining analysis which displays short internal branches and low bootstrap values throughout its entire base structure. This uncertainty may have arisen for a number of reasons. Molecular evidence for phylogeny can be attenuated by multiple substitutions at single nucleotide sites, with transitions being likely to become saturated before transversions (Hillis *et al.*, 1996, Harris *et al.*, 1998). However, the low level of differentiation between samples in this situation would indicate that saturation has not taken place. The most probable reason for lack of resolution is that the sequence investigated is to some extent inappropriate for examining closely related groups, having too few informative sites to provide a statistically useful estimation of intraspecific relationships (Harris *et al.*, 1998). Melville and Swain (1998) examined cytochrome *b* variation within all *Niveoscincus* species and found sequence differences in the range of 6.8 to 23.1%. Indeed within *N. metallicus* from Type 1 populations, they observed variation in the order of 9.8 to 12.1%. These values are significantly higher than those observed for 16S rRNA in my study. Sequence divergence within groups for this gene ranged from 0.0 to 1.4% while between group variation levels were between 0.87 and 2.83%. However, despite this apparent low sequence divergence, there was still strong support by bootstrapping for some nodes, indicating that enough variation is apparent to resolve some relationships.

One factor which may provide some hindrance to the resolution of the neighbour-joining tree is the vast difference between the outgroup, *P. entrecasteauxii*, and the ingroups . The smallest value for differentiation between the outgroup and the most similar ingroup is 11.5% in comparison to the most distinct ingroups which were only 2.83% different. Swofford *et al.* (1996) indicated that systematic error is expected to be worse in distance analysis when large branches are present in the analysis. However, they state that to reduce this problem the use of outgroup sequences should be kept to a minimum when using pairwise distance methods. While the selection of a sister species as an outgroup may have influenced the topology of the tree produced, the general agreement between the distance and parsimony analyses suggests that this is unlikely.

If the above possibilities are excluded, it becomes more probable that the lack of strong support in the topology of the base of both analyses results from a period of rapid speciation occurring in the recent past, probably during the climatic extremes of the Pleistocene. This has been suggested in other studies (Freisen *et al.*, 1996; Lara *et al.*, 1996), including one where sequence data from the 12S rRNA gene failed to resolve relationships in the scincid lizards of the genus *Leilopisma* (Hickson *et al.*, 1992). Harris *et al.* (1998) also provided the example of the South African sand lizards (*Meroles* spp). This group was morphologically variable but showed little DNA variation, and this was attributed to rapid speciation in a harsh environment.

16S rRNA proved to be somewhat less variable than has previously been shown for the protein coding gene cytochrome *b* within this species (Melville and Swain, 1998) Melville and Swain (2000b) re-examined the species of *Niveoscincus* using cytochrome oxidase and found substantially less variation (4.0 to 12.6% between species). They also observed an intraspecific level of variation within *N. metallicus* of 0.7% for animals from Orford and Clarence lagoon (Type 1 sites). This value is identical to the largest within group variation observed between Type 1 animals in my study (Table 3.3) indicating that 16S rRNA is perhaps only slightly less variable than cytochrome oxidase. The majority of studies on phylogeny in reptiles focus on the protein coding gene cytochrome *b* or the rRNA genes 12S and 16S (Caccone *et al.*, 1997). Slower rates of evolution in rRNA genes have been well documented (Caccone *et al.*, 1997); however, Hedges *et al.* (1991) observed little variation in rates between cytochrome *b* and 12s rRNA in an examination of xantusiid lizard biogeography.

Many authors assume a 2% divergence rate per million years (Brown *et al.*, 1982; Wilson *et al.*, 1985; Thorpe *et al.*, 1994; Melville and Swain, 1998, 2000b) for mt DNA (averaged across sequence and RFLP data). Molecular dating, such as this, has always proven appealing because it may be used when no other information, such as stratigraphy and paleontology, are available for time estimates (Caccone *et al.*, 1997). However, the use of molecules as time indicators relies on assessing whether molecular divergence is linear over time. This issue has been controversial since its introduction by Zucherkandl and Pauling (1965) as the molecular clock or rate-constancy hypothesis (Caccone *et al.*, 1997). A number of studies have challenged this hypothesis, showing that nucleotide substitution rates vary dramatically within gene regions and among divergent taxonomic groups, in

both the nucDNA and mtDNA genome (Wu and Li, 1985; Powell *et al.*, 1986; Bulmer *et al.*, 1991; Martin *et al.*, 1992; Caccone *et al.*,1997; Keogh *et al.*, 1998). However, the concept has been generally accepted for comparisons among closely related species or subspecies; because they share a recent common evolutionary history, such groups are unlikely to differ in aspects of their biology which may influence nucleotide divergence (Hillis *et al.*, 1996).

Published evolutionary rates for mt DNA ribosomal genes in both endotherms and ectotherms are 0.5 to 1% / million years for transitions and transversions (Meyer and Wilson, 1990; Mindell and Honeycutt, 1990; Hillis and Dixon, 1991; Meyer, 1993; Caccone *et al.*, 1997). Despite this, the substitution rate of 1 to 2% per million years, based on primate mtDNA (Brown *et al.*, 1979, 1982), continues to be used for vertebrates and has recently been used in other studies on molecular biology of lizards (e.g. Thorpe *et al.*, 1994; Melville and Swain, 1998). This estimate was also used successfully by Melville and Swain (2000b) in their study of cytochrome oxidase, which has been shown to have evolved perhaps only slightly faster than 16S rRNA (see above). Thus, it is likely that sequence divergences in *N. metallicus* for 16S rRNA is somewhere around the 1 to 2% / million year level.

The current biogeographical patterns in *N. metallicus* may be explained by the vicariance hypothesis, which requires some restriction of gene flow between populations. Vicariant biogeographic analyses focus on congruent patterns among monophyletic assemblages of species or subspecies, addressing the role of past geological events in shaping present day distributions (Lamb *et al.*, 1992). This type of phenomenon is obvious in Tasmania where the flora has been affected by the Pleistocene glaciations (Greenwood, 1994). Melville and Swain (1998, 2000b) have suggested that ancestral populations of *Niveoscincus* were subject to expansions and contractions during the Pleistocene glaciations in association with the habitats in which they occurred.

A rate of divergence of 1 to 2 % suggests that the differentiation observed within *N. metallicus* also occurred at some stage during the past 1 million years, probably starting during the early Pleistocene. Towards the end of the Pliocene the seasonal pattern in Tasmania changed from summer to winter precipitation. This change, coupled with the beginning of the glacial-interglacial oscillations of the climate in the early Pleistocene,

would have created massive changes in the structure of plant communities (Jackson, 1999), a situation promoting divergence in many species. Four glacial episodes have now been identified during the Pleistocene, the last or Margaret Glaciation (30-13 ka), the Henty Glaciation (>140 ka), the Moore Glaciation (>200 ka) and the Linda Glaciation (>730 ka). This nomenclature was used by Fitzsimons and Colhoun (1991) and Jackson (1999). The earlier glaciations were more extensive than the more recent ones. For example, during the Linda Glaciation, which occurred in the Early Pleistocene or Late Pliocene, an ice sheet of about 6000 km^2 occupied the central-western highlands, extending from the Great Lake to the valley of the Huskisson (Kiernan 1990). Large valley glaciers also extended to near sea level in the Forth and Mersey Valleys (Jackson, 1999; Colhoun *et al.*, 1996; Kiernan, 1996). This level of glaciation may have formed a boundary separating the northwest corner of Tasmania from the remainder of the island and may have represented an almost impenetrable biogeographic barrier. Even during the most recent glacial period, eucalyptus forest would have been restricted to the northwest and northeast coasts (Kirkpatrick and Fowler, 1998), with extensive areas of true alpine habitat covering much of central and western Tasmania. The topology of both the parsimony and distance matrix trees indicates that northwestern *N. metallicus* were the first group to diverge from the ancestral type. Indeed Type 3 animals are the most distinct group formed, being most similar to Type 1 animals (about 1.5% divergence). It therefore appears probable that this group diverged first during the start of the glacial fluctuations characteristic of the Early Pleistocene.

The next major split between groups differs somewhat between the parsimony analysis and the distance matrix tree. The split is, however, only weakly supported in both tests. The difference between the two trees is that Type 2 (South Cape) animals occupy different positions. In the parsimony bootstrap tree (Figure 3.7) South Cape animals are grouped with Type 1 lizards. Geographically this would be expected. Conversely, the neighbour-joining tree (Figure 3.8) groups South Cape animals with Type 4 and 5 lizards from northeast Tasmania and Victoria. This pattern of evolution seems unlikely, due to the large distance between these populations. In addition the tree displays very short internal branches in this section of its topology, indicating potential difficulties in resolving their relationships. Consequently, I believe it more appropriate to discuss possible vicariance events leading to divergence in terms of the more realistic parsimony analysis.

Large level glaciations were not evident in the northeast of Tasmania during the Pleistocene, although a small glacier would have been present on Ben Lomond (Jackson, 1999). However, Kirkpatrick and Fowler (1998), in a study attempting to predict refugial sites for flora in Tasmania during the Late Pleistocene, demonstrated that eucalypt forest may have been restricted to the northeast and northwest of Tasmania during the height of the last glaciation. It could be expected that this may have also occurred during earlier glaciations. Large regions surrounding this northeastern corner would have been true alpine habitat, with areas of grassland in the far north. This may have provided a boundary to gene flow between Type 4 and Type 1 populations. As the preferred habitat of this species was restricted to refugia, the animal may have been restricted to habitats in the northeast, and pockets of habitat in the southeastern corner of Tasmania. Mesibov (1996) identified the northeastern corner of Tasmania, and specifically Plomley's Island (Mesibov, 1994), as perhaps the most obvious bioregion in Tasmania, in terms of invertebrate fauna.

Mesibov (1996) also reported that a number of characteristic species of invertebrate are found only in the far northeast and on Flinders Island and Victoria. This observation supports the high degree of relatedness evident between Type 4 and Victorian Type 5 animals in both the parsimony and neighbour-joining trees. The last glaciation lowered the sea level by about 120 m, indicating that bridging of Bass Strait would probably have occurred during all of the glacials (Jackson, 1999). During the period 24-16 ka the sea level would have been lower than –70 m providing two land bridges to Victoria, one through Flinders-Deal Islands to Wilsons Promontory and one through Hunter-King Islands to the Mornington Peninsula (Jackson, 1999). A large shallow lake would have occupied most of central Bass Strait (Blom, 1988). Assuming that central Tasmanian glaciers separated northwestern and northeastern populations of *N. metallicus*, then both populations would still have had access to Victoria through these land bridges. However, the tree line on the western side of Tasmania during this period would have dropped out at the present day sea level (Jackson, 1999) and conditions may have been unfavorable on the Hunter-King Island land Bridge. This may have prevented northwestern populations from interacting with Victorian animals. Kirkpatrick and Fowler (1998) demonstrated that eucalypt forest would have been present over almost all of what is today Flinders Island, and this land bridge would possibly have been much more hospitable than the western

one. Horwitz (1988) also provided an example of a hypothetical rise and fall in sea level associated with climatic fluctuations evident in the early Pleistocene. During this period, based on present day depth contours of Bass Strait the sea would have entered the Strait between King Island and Cape Otway, gradually splitting northwestern and Victorian populations. With a slow rise in the sea level (of no more than 1 m per 70 years (De Deckker, 1986), the northeastern Tasmanian population would have remained in contact with Victorian populations for extensive periods while a barrier of water and ice enclosed the northwestern populations. Thus, it is probable that Type 4 and Type 5 populations were subject to gene flow at many stages during the Pleistocene, while Type 3 animals were isolated.

The final divergence that requires some level of explanation is the segregation of Type 1 and Type 2 animals in the far south of Tasmania. Evidence for glaciations in this area of the state is scant and somewhat unreliable. However, it is known that the summits of the Ironbound Range, Mt La Perouse, Pinders Peak, Mt Victoria, Mt Bisdee and Adamsons Peak were all subject to glacial activity (Derbyshire *et al.*, 1965). The intensity of glaciation in this area is unknown; but it is possible that it may have been sufficient to isolate populations of *N. metallicus* during the Pleistocene. Kirkpatrick and Fowler (1998) indicated that, at the height of the last glaciation, much of this area may have been alpine habitat. However, patches of eucalypt forest may have remained to act as refugia.

The final question that must be addressed in a situation involving the disclosure of a number of subtypes within a recognized species is the taxonomic status of these populations. I do not propose any formal change to the taxonomy of *N. metallicus* in this thesis for a number of reasons. Firstly, although the RFLP analysis and the sequencing analysis both revealed species sub-structuring, with little intra-group variation, divergence levels were not large. Also, my work is based solely on the 12S rRNA and 16SrRNA regions. Due to the conservative nature of these regions, further sequencing work is required, specifically examining the cytochrome *b* region of the mtDNA fragment. Secondly, although sampling was extensive, further collections from both Tasmania and Victoria would be helpful in explaining observed geographical patterns. Finally DNA analysis alone is not sufficient to segregate populations into subspecies in the absence of any morphological support. This does not imply that no variation is present across the range examined, but simply that it has not been examined. Nevertheless the question of

taxonomic status in this species is not trivial. Populations are clearly allopatric but geographically proximate, perhaps overlapping in some areas (e.g. Ben Lomond). To determine whether separation of these groups satisfies currently accepted species concepts (Rose and Selcer, 1989; Frost and Hillis, 1990; Zamudio *et al.*, 1997) is therefore of importance.

The results from my work suggest that a single parental *N. metallicus* type gave rise to five modern subtypes during the extensive climatic fluctuations experienced in the Pleistocene. The timing of these events is in accord with previous work conducted by Melville and Swain (1998, 2000b), who have proposed that the genus *Niveoscincus* first appeared in the Tertiary and subsequently diverged in the Early Pleistocene. In future research I intend to examine cytochrome *b* sequences and morphological evidence for segregation of populations in this species. I also hope to investigate population genetics across the boundaries of the regions identified, and to study island populations of *N. metallicus* throughout Bass Strait to shed further light on possible mechanisms of speciation in this group.

CHAPTER FOUR

Microhabitat occupation and basking site selection in *Niveoscincus metallicus*

4.1 Introduction

Habitat and microhabitat use have received a great deal of attention from reptile ecologists (Adolph, 1990), usually in the context of resource partitioning and interspecific competition (Schoener, 1977; Toft, 1985). A variety of factors influence how animals use their habitats and microhabitats. Among reptiles, the most important of these are temperature, food distribution, intra- and inter specific interactions and predation (Smith, 1996), all of which vary spatially. In particular, interactions between ectotherms and their thermal environment can restrict activity and habitat use (Christian *et al.*, 1983; Huey *et al.*, 1989; Adolph, 1990; Vallejo *et al.*, 1995; Blazquez, 1996; Melville and Swain, 1997a, 1997b; Christian, 1998) and may conflict with other factors including food acquisition and predator avoidance (Huey, 1982; Downes and Shine, 1998).

Because thermal microclimates vary spatially lizards may use specific habitats or microhabitats at different times of the day, or at different locations (e.g. elevations), to maintain preferred body temperatures (Carrascal and Diaz, 1989; Aldoph, 1990; Castilla and Bauwens, 1991; Smith, 1996). Lizards compensate for environmental extremes primarily through behavioral shifts, including the selection of specific microhabitats (Hertz and Huey, 1981; Marquet *et al*, 1989; Adolph, 1990; Carracal *et al*, 1992; Melville and Swain, 1997a, 1997b). Thus, at high altitude a lizard may be restricted to only the warmest microclimates and suitable microhabitats may have increased value because of their reduced availability (Hertz and Huey, 1981). However, while a lizard must contend with extremes in its physical environment, that same environment can also be exploited in order to optimise the animal's physiological state (Huey and Stevenson, 1979). For example, such behaviour may result in more efficient avoidance of predators (Christian and Tracy, 1981). In addition, patterns of habitat use can influence life history and demographic traits both within (Smith, 1995), and between populations (Grant and Dunham, 1990) by affecting the abiotic and biotic environment. Consequently, it is

important to consider how individuals use their environment if the potential for interindividual differences in habitat occupation to result in life history variation is to be understood (Smith, 1996). This is especially true for species that occur over large geographic and/or altitudinal ranges, since these are likely to show the greatest variation in such relationships.

Many lizard species have relatively narrow ranges of active body temperatures that correspond to various physiological optima (Bennet, 1980; Huey, 1982, Adolph, 1990). In temperate climates a strategy of precise thermoregulation requires flexible use of the habitat if a species is to cope with temporal or geographic variation in the thermal environment (Adolph, 1990). If this does not occur, opportunities for activity may be severely curtailed. It is therefore of interest to examine habitat use among populations of widespread species, to determine the extent of behavioural plasticity.

An intrinsic problem encountered when quantifying the microhabitat characteristics preferred by shuttling heliotherms such as *Niveoscincus metallicus* is that the animals are generally only visible when basking (Melville, 1994). This is common among cryptic species (Barbault and Maury, 1981). The basking site selected by a lizard is, therefore, often the only consistent indicator of microhabitat use and, consequently, is commonly used by investigators (Moermond, 1979). This approach is based upon the premise that the thermoregulation of lizards in temperate climates is reliant upon the availability of suitable basking sites.

Microhabitat use by *N. metallicus* was previously investigated at a sub-alpine site on Mt Wellington (Melville and Swain, 1997); however, this research was conducted as part of a study looking at spatial separation between this and a related species, *N. microlepidotus*. Other than this, existing descriptions of habitat use for this and other Tasmanian species are mostly qualitative, and generalised (Rawlinson, 1974; Hutchinson *et al.* 1989) although Melville and Swain, (1999) used a quantitative approach in their review of habitat associations and biogeography of snow skinks. The aim of my investigation is to expand on current knowledge of habitat occupation in *N. metallicus*, by examining inter-populational variation on two separate spatial scales. Morris (1987), Wiens (1989) and Rubio and Carrascal (1994) have all stressed the importance of spatial scale in the analysis of habitat preference. However, this has received little attention in studies involving

reptiles. Habitat preference was examined at the local scale (3 m radius around animal-overall habitat elements) and at the focal scale (basking site characteristics) in *N. metallicus* using multivariate techniques. These two scales allowed separate examination of differential habitat use between sites (both scales) and habitat selection within sites (local scale only). The study examines if (1) animals actively select specific cryptic elements within a habitat or occupy sites in a random fashion; (2) lizards show any geographic pattern to their habitat occupation associated with altitude and latitude; and (3) whether factors other than thermal environment (e.g. predation) may explain observed results.

4.2 Materials and Methods

4.2.1 Field Methods

Observations were made at the four previously described field sites (Chapter 2) during the summers of 1998 and 1999. Data were recorded by starting from a randomly determined location and moving slowly back and forth across the site to ensure coverage of the entire range of microhabitats available. When an undisturbed adult lizard was sighted basking the position and sex were recorded immediately. Wherever possible lizards were captured by noose and the following information was recorded.

Focal scale (at basking site):

1. level of shade on lizard (% cover) (SH);
2. basking surface ((1) large rock, (2) rock and vegetation, (3) thin vegetation and dirt, (4) vegetation and sticks, (5) branches, (6) logs)(BS);
3. distance to nearest shelter (cm)(DS);
4. distance to nearest vegetation (cm)(DV);
5. major substrate component ((1) scree, (2) small rocks/vegetation, (3) litter/vegetation, (4) branches/vegetation, (5) logs)(ST);
6. vegetation cover over lizard ((1) = none to (5) = full, complex)(VC);
7. height of vegetation (cm)(VH);
8. type of vegetation (Structure (1) = simple grasses to (5) solid logs and branches)(VT);
9. basking height (cm)(BH).

Once the above information was determined, an area of 3 m in diameter was estimated around the lizard's basking site. From this area the following information was obtained.

Local scale (3 m diameter - all values recorded as presence by percentage):

1. gravel;
2. grass;
3. fallen leaves;
4. rocks (only used in NP-Manova);
5. soil;
6. tree trunks;
7. tree branches;
8. vegetation less than 25 cm height;
9. vegetation between 25 and 50 cm;
10. vegetation between 50 and 100 cm;
11. vegetation between 1 and 2 m;
12. vegetation above 2 m height;
13. rocks less than 25 cm in diameter;
14. rocks between 25 and 50 cm diameter;
15. rocks between 50 and 100cm diameter;
16. rocks above 1 m diameter;
17. slopes less than 20 degrees.

I also estimated the availability of microhabitats at each study site by measuring the above variables in an area with a center that was randomly determined by stopping every 15 minutes during lizard collections. This provided a means of examining selection of microhabitats at each site.

4.2.2 Statistical Analysis

4.2.2.1 Focal scale

A principal components analysis (PCA) was used to reduce the nine focal scale variables to a smaller number of uncorrelated components that describe the underlying dimensions

in the basking sites occupied (FACTOR procedure of SYSTAT, Wilkinson *et al.*, 1992). The principal components were extracted from the correlation matrix of the raw data. This matrix has the advantage of being independent of the scale of variables measured (Tabachnick and Fidell, 1989). Data were not transformed prior to the analysis as some of the values for the distance to vegetation and shelter were very small and I wished to reduce the influence of less common habitat components in the analysis. Thus it is possible that the assumption of linearity was violated (James and McCulloch, 1990). However, departures from linearity are not usually great enough to invalidate a PCA (Pimentel, 1979). The number of principal components (PCs) utilized in the analysis was determined by using the scree test of eigenvalues plotted against factors, maximizing the adequacy of extraction. PC axes were named by the correlation of the original variables to the PC; correlations with absolute values of >0.5 were considered significant (Tabachnick and Fidell, 1989). Interpretation of PCs was straightforward, so there was no requirement for rotation of the data.

Subsequent to PCA I performed a univariate ANOVA analysis on the separate PC axes using the MGLH procedure of SYSTAT (Wilkinson *et al.*, 1992), in order to determine if there was any significant separation of populations along the axes. Even though sample sizes between populations were uneven, there was no violation of statistical assumptions (Tabachnick and Fidell, 1989), and sample sizes were large enough to ensure multivariate normality. Using the discriminant function analysis, PC scores (means and standard errors) for populations were determined.

4.2.2.2 Local Scale

A Bray Curtis similarity matrix was generated using data from all four populations. Site similarity was tested using an analysis of similarity (ASIM module; 1000 randomizations) based on the Bray Curtis matrix. The similarity matrix was ordinated in three dimensions using 30 random starts in the SSH (semi-strong hybrid multidimensional scaling) module. Attributes of each habitat were correlated with the three ordination axes using the PPC module. Correlations were tested for significance using 100 Monte-Carlo randomizations (MCAO module). All analyses were conducted using PATN (Belbin, 1995).

I used a two-way crossed (orthogonal) non-parametric MANOVA to again test for site similarity (Anderson, 1999). This analysis uses a non-parametric method for testing ecological multivariate hypotheses. It allows correct calculation of statistics for crossed factors, which are fixed or random in design. Factors tested in this design were site (random effect) and treatment (i.e. two fixed levels – randomly chosen area or site containing basking lizard).

The analysis provides a partitioning of multivariate variability in the data according to particular factors in any two-way ANOVA design on the basis of any of several different distance measures. In this case I used a Bray-Curtis dissimilarity measure. Data were not transformed prior to the analysis. This method also allows further analyses of particular terms by providing multivariate pair-wise *a posteriori* tests. However, this test is not corrected for experimental-wise error rate. This means that if one selects a significance level of 0.05 for the tests, then a significant result should be expected in 1 out of 20 tests by chance alone. Thus the onus is on the user to interpret results in a conservative way.

4.3 Results

4.3.1 Focal scale

The principal component analysis (PCA) was first performed separately on both sexes of animals from each site; however, since no significant differences were found between sexes the data were pooled.

The PCA that incorporated two factors accounted for 56.4% of the variance of the raw data (Table 4.1). The number of factors used in the analysis was determined using a scree test, where a change in slope indicates the final factor which should be employed. The first PC axis accounted for 39.9% of the variation in the raw data. It yielded a strong positive correlation with basking surface, substrate type, vegetation cover, vegetation height and vegetation type. A strong negative correlation with distance to shelter and distance to vegetation was also evident (Table 4.1). Thus, the PC1 axis describes a basking site type in which high PC scores indicate a preference to bask on wood rather than rock, to bask on more complex substrates, to shelter under vegetation, and to use more complex vegetation associations. Animals displaying high PC1 scores also bask closer to shelter and to ground

level vegetation. Negative PC scores denote more open, rocky areas, with little or no cover and greater distances to shelter.

The second PC axis accounted for 16.573% of the variation in raw data. It displayed a positive correlation with distance to shelter, vegetation height and basking height. Thus high PC scores on this axis indicate animals basking further from shelter, under high vegetation and basking higher above the ground.

Table 4.1 Correlations between original environmental variables with the first two PC scores for *N. metallicus* populations from all field sites.

		PC1	PC2
Correlations with PCs		BS 0.657 DS –0.535 ST 0.754 DV -0.633 VC 0.818 VH 0.671 VT 0.763	DS 0.503 VH 0.618 BH 0.749
Percent of Variance		39.855	16.573
	n	Site Score (Mean ± SD)	Site Score (Mean ± SD)
Mt. Wellington	67	-0.72 ± 0.72	0.10 ± 0.87
Clarence Lagoon	57	-0.61± 0.92	0.32 ± 0.97
Orford	74	0.30 ± 0.87	0.01 ± 0.96
Hobart	103	0.59 ± 0.80	-0.25 ± 1.07

Of the original 301 lizards recorded, 4 appeared to represent outliers from the data set; however, due to the natural variation in this type of information, there was no reason for removing these animals from the analysis. Assumptions of linearity and were not violated for the remaining 297 lizards.

A discriminant function analysis revealed intraspecific variation in the PC scores on both PC1 and PC2 (Table 4.1). The highest scores on PC1 were yielded by animals from Hobart and Orford and the lowest scores occur for the Mt. Wellington and Clarence Lagoon populations. There were significant differences between sites on the PC1 axis

($F_{3,297}$ = 48.617, P<0.001). On PC2 the highest score is evident for animals from the Mt. Wellington and Clarence lagoon sites. Again significant differences were evident on this axis ($F_{3,297}$= 4.534, P=0.004).

A Tukey HSD multiple comparison indicated that there is grouping of populations based on basking site utilization (Figure 4.1). High altitude sites (Mt. Wellington and Clarence Lagoon) were not significantly different from each other on PC axis 1. The same was true for low altitude sites (P>0.05). However, all low/high altitude combinations displayed significant variation on this axis (P<0.001). Only the Clarence Lagoon and Hobart sites were significantly different on PC axis 2 (P=0.002). Thus basking site occupation appears to be most reliably predicted in this analysis using the PC1 scores, with high altitude animals basking more often on rock and in more exposed microclimates.

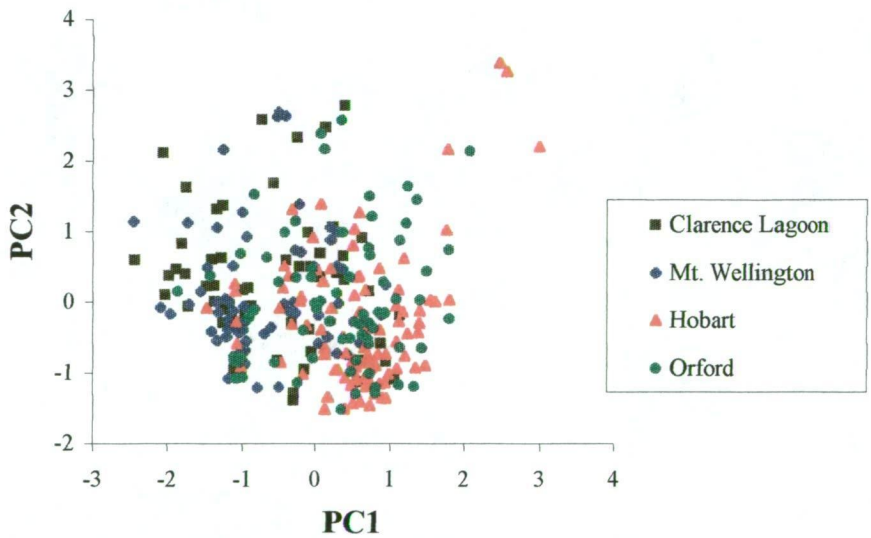

Figure 4.1: First two principal components (PCs) of the analysis of basking site selection by *N. metallicus* individuals from four populations around Tasmania.

4.3.2 Local scale

Initially, I examined local scale data using PCA, however, this technique was unable to discern differences between sites; however, professional advise (Barmuta, *pers com*) indicated that semi-strong hybrid multidimensional scaling was a more appropriate technique to examine data. Therefore the analysis continued using this procedure. The

level of variation observed between sites was significantly larger than that occurring within sites (ASIM statistic < 0.001). Thus in the local scale analysis at least one site was dissimilar to others in terms of the habitat adopted by lizards. Ordination of the data revealed that variation across sites was continuous with no site occurring in isolation (Figs. 4.2 and 4.4). Despite this, trends in habitat occupation between sites are evident. All habitat components displayed significant correlation with ordination axes at the 0.01 level (MCAO module).

The trend evident across ordination axes 1 and 2 is weak. Animals from Mt. Wellington tend to occupy the top, central and right positions on the graph. These correspond to areas with significant rock cover, and mid level vegetation. Clarence Lagoon animals display little in the way of any obvious trends on these axes, with animals occupying most of the graph surface. Some clustering in the lower central portion indicates an association with high level vegetation and branches. Hobart lizards occupy a central position on the graph, indicating an association with low level vegetation and grass. Orford animals follow a pattern similar to that observed for Mt. Wellington individuals, occurring in the central right of the graph. These animals are primarily associated with low to mid level vegetation.

Figure 4.4, displaying the plot of ordination axes 1 and 3, more clearly differentiates between sites. Animals from high altitude sites tend to occupy the lower right area of the graph. These sites are associated with rocks, dead branches, mid level vegetation and gravel. Hobart animals cluster in the top left of this graph. They occupy a habitat characterized by trees and low vegetation (including grass). Orford animals are found again across the entire surface of the graph; however, they cluster in the top of the graph, indicating an affinity for areas with low vegetation. Despite this, some animals are found in areas dominated by exposed rock. These trends are best examined in combination with vector plots (Figs. 4.3 and 4.5), that reveal the importance of specific habitat components on ordination axes.

The non-parametric two way MANOVA indicated that there was a strong interaction effect between site and treatment (lizard present or randomly determined site) ($F_{3,392}$ = 6.3312, P = 0.001). Pair-wise *a posteriori* comparisons were determined for all possible combinations of this interaction. Tests among groups in treatment for all levels of site

indicated that at the Mt. Wellington site animals displayed strong differences between random and selected habitats ($P = 0.0002$). At Clarence Lagoon, selection was supported, but not strongly ($P = 0.057$). At both low altitude sites, no selection was evident ($P = 0.9992$ and 0.9588 for Hobart and Orford respectively).

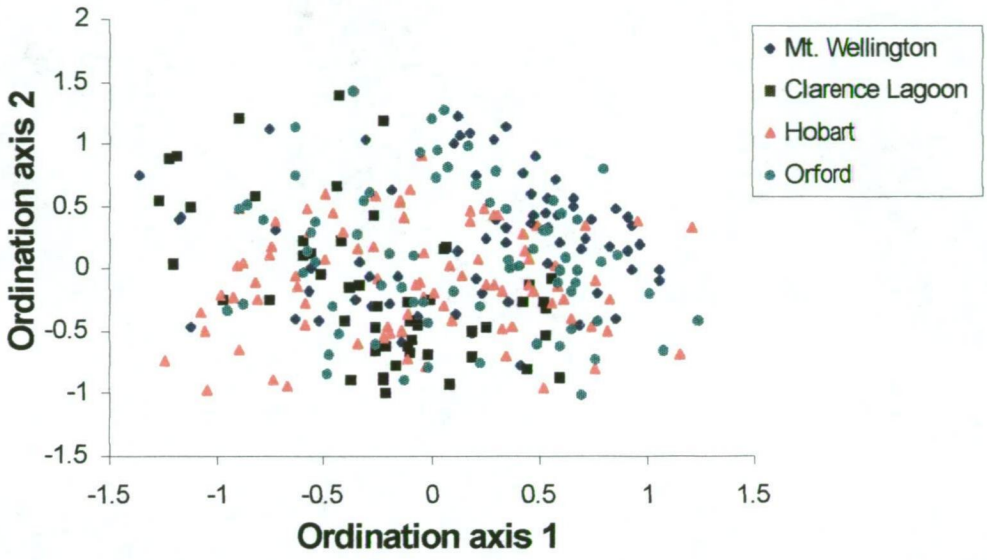

Figure 4.2: Plot of ordination axes 1 and 2 showing continuous variation between sites in terms of habitat selected by *N. metallicus* individuals from four populations around Tasmania.

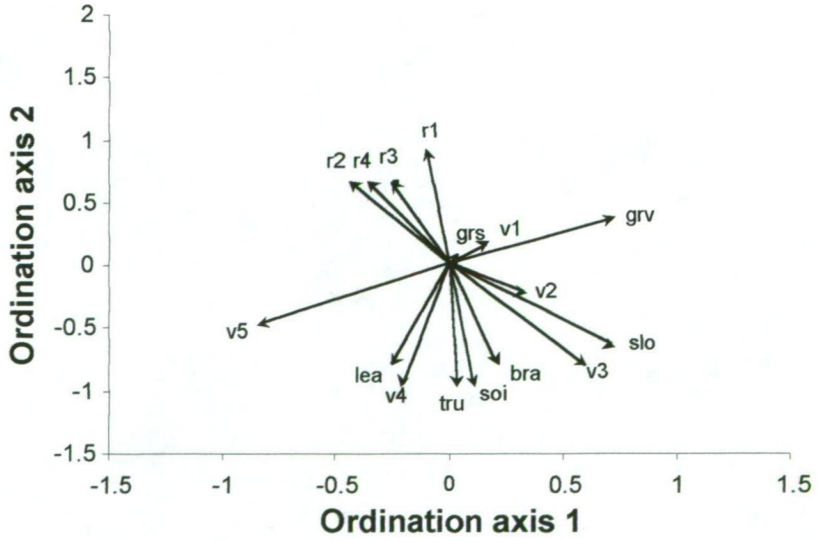

Figure 4.3: Plot of the vectors on ordination axes 1 and 2 produced from a principal axis correlation following semi-strong hybrid multidimensional scaling of all four sites. Arrow length is proportional to the correlation coefficient for that habitat attribute.

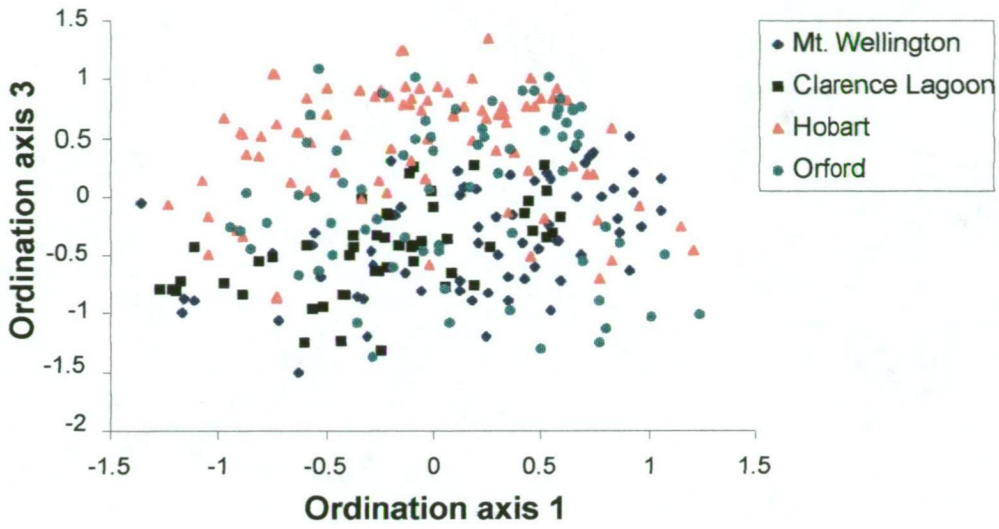

Figure 4.4: Plot of ordination axes 1 and 3 showing continuous variation between sites in terms of habitat selected by *N. metallicus* individuals from four populations around Tasmania.

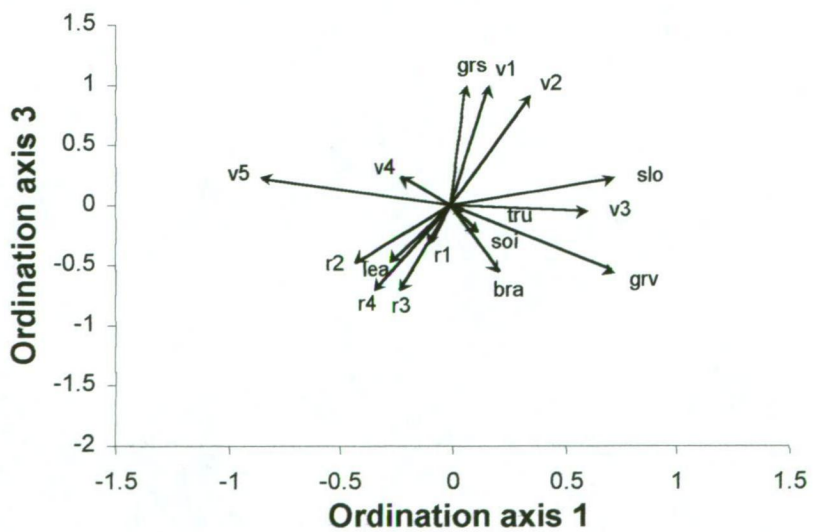

Figure 4.5: Plot of the vectors on ordination axes 1 and 3 produced from a principal axis correlation following semi-strong hybrid multidimensional scaling of all four sites. Arrow length is proportional to the correlation coefficient for that habitat attribute.

Tests among populations at the lizard present level of treatment indicated that all sites were significantly different from the Orford population in terms of habitat selected ($P < 0.05$). All other combinations of sites did not differ significantly. Finally, an examination of site differences for randomly selected areas indicates that only two groups of sites are not significantly variable. Clarence Lagoon and Hobart appear structurally to be similar ($P = 0.7332$) while Mt Wellington and Orford also appear similar ($P = 0.264$). Thus, structurally, sites group into open rocky (Mt Wellington and Orford) and forested (Clarence Lagoon and Hobart) habitats. All other combinations of sites were significantly different ($P < 0.001$).

4.4 Discussion

All species exhibit a non-random selection of microhabitats and a preference for a particular set of environmental and structural factors. The selection of microhabitat characteristics by lizard communities has been reported in many studies (see Paulissen, 1988 for early examples). In the current study, habitat utilization was investigated at two levels, to determine to what extent *N. metallicus* utilizes long term behavioural responses (microhabitat selection) to compensate for variation associated with altitude. Attempts to provide qualitative descriptions of microhabitat occupation in small shuttling heliotherms are often complicated by the fact that animals are only visible when basking (Melville and Swain, 1997a). I chose to define microhabitat utilization in *N. metallicus* in this investigation through the use of basking sites. This was because these sites are a dominant characteristic of an animal's home range, and because, in this species, thermoregulation occupies a large portion of the activity window in all seasons (Melville and Swain, 1997b). Numerous other investigations have also used basking site as an indicator of microhabitat use (Moermond, 1979, Hertz and Huey, 1981, Hertz *et al.*, 1994, Vallejo *et al.*, 1995, Melville and Swain, 1997a).

My study indicates that basking site selection (focal scale) is strongly related to vegetation, substrate and cover. Site differences were detected along both PC axes 1 and 2 (Figure 4.1). Generally sites separated into high and low altitude clusters with animals from high altitude sites displaying a preference for rock basking surfaces with little or no vegetation cover. These animals also basked further from shelter. Low altitude animals

basked generally on wood, with complex vegetative cover and closer to refugia. There was also a weak tendency for high altitude animals to bask higher above ground level, generally related to their preference for rock basking surfaces. Other studies of lizard communities have found very similar factors to be involved in the selection of microhabitat, even in very different habitat types. For example, Shenbrot *et al.* (1991) indicated that substrate and vegetative characteristics were the most significant factors in determining the selection of microhabitat by some small desert lizards. Martin and Salvador (1995) also indicated that substrate type, specifically rock, was a major determinant of population density in an Iberian rock lizard, *Lacerta monticola.* Numerous studies have also reported that structural characteristics such as vegetation and substrate type are of primary importance to temperate climate lizard species (Dent and Spellerberg, 1987; Paulissen, 1988; Patterson, 1992; Brown and Nelson, 1993). Likewise, studies of habitat use across altitudinal gradients have reported that microhabitat selection can be of major importance in adapting to high altitude environments (Marquet *et al.*, 1989; Adolph, 1990; Spencer and Grimmond, 1994), a trend observed in *N. metallicus.*

Investigation of microhabitat selection at a local scale modified the conclusions obtained from examination of focal scale results. Multidimensional scaling indicated that there is little separation of sites along axes 1 or 2 (Fig. 4.2). However, much stronger differentiation was apparent on axis 3 (Fig. 4.4). Again, the indication was that animals from low altitude tend to occur in areas of complex vegetation cover while high altitude animals are more prevalent in regions denoted by rock, gravel and more open structural elements. The non-parametric MANOVA provided more information on the separation of sites at this scale. Initially this analysis revealed that selection of microhabitat was occurring only at the two high altitude sites. Animals at Hobart and Orford appeared to show no selection of habitat beyond that associated with the selection of suitable basking sites. Clarence Lagoon and Mt Wellington individuals, however, showed evidence for selection of specific elements of habitat. Selection is obvious from direct observation at the Mt Wellington site with animals occurring in open forest areas and never on boulder scree (see Melville and Swain, 1997a). However, at Clarence Lagoon support for this conclusion was not quite significant ($p=0.057$). Animals here appear to select more open areas than those randomly available. Lizards were never observed in the more structured forest zones, but tended to occur in areas with little or no tree cover. At both sites, animals select areas of partial tree cover and mixed level ground cover.

The comparison of randomly selected areas between sites revealed that microhabitats separate according to complexity of tree cover and vegetation rather than location (i.e. altitude). Thus at this scale a different relationship between sites becomes evident. If examination is restricted to those areas with lizards present, it is apparent that the Orford site was significantly different to all other sites. This is predictable when comparing Orford to the more forested sites; however, the difference evident between Orford and Mt. Wellington supports the argument that selection of microhabitat by animals is occurring at the Mt. Wellington site. Animals from Mt. Wellington and Clarence Lagoon appeared to select similar habitats at the scale chosen (3 m diameter), despite the sites differing in microhabitat availability. Thus, although these sites provide quite distinct microhabitats, altitude appears to impose some restriction on finer scale habitat selection. Animals from Hobart and Orford used habitat differently, but this was predictable since the sites were distinct in the random analysis and no selection of microhabitat was evident in either population. The only result difficult to explain is the finding that Mt. Wellington and Hobart sites were not significantly different (MANOVA) in terms of areas with lizards, but did appeared to be distinct in the random site analysis. This may indicate that factors other than altitude and habitat complexity (e.g. food availability, social interactions, or predation) are important at this scale, or it may represent a type 2 error in the analysis. All other relationships between field sites at this scale support the findings of the focal scale investigation, or can be explained in terms of microhabitat complexity and broad scale selection at each site.

Thermal biology and habitat usage are interrelated because thermal microclimates vary spatially (Adolph, 1990). Habitat use by a lizard species reflects integration between the microhabitats that are thermally acceptable and those that are suited to its morphology and behaviour (Adolph, 1990). The body temperature that a lizard is able to maintain has an important influence on many physiological processes and life history traits (Adolph and Porter, 1993). *Niveoscincus metallicus* appears to be able to meet its thermal requirements by adopting a strategy of flexible microhabitat use across its range. At high altitude it tends to utilize relatively open rocky habitat. This preference has a number of advantages. By occupying more open, rocky microhabitats, *N. metallicus* may be able to maximise its use of available sunlight and the heat storage properties of the substrate. However, *N. metallicus* does not utilize rock scree, the most open habitat available at high altitude. At

both high altitude sites, other morphologically more specialized species occupy these habitats (Melville and Swain, 2000a; personal observations). Instead *N. metallicus* occupies the most open habitats that its physiology and morphology permit. *Niveoscincus metallicus* is a ground dwelling cryptic species, found in a wide range of habitats (Rawlinson, 1974; Hutchinson and Schwaner, 1991; Melville and Swain, 1999b). This investigation reveals that, at its altitudinal extremes, this species is still associated with protective microhabitats, but utilizes the most exposed sections of these habitats.

Size, and subsequent high surface area to volume ratio, may also limit the distribution of *N. metallicus* to closed habitats at high altitude. Occupation of densely vegetated areas would reduce exposure to the cold winds that blow much of the time (Melville and Swain, 1997a). Marquet *et al.*, (1989) found that smaller species of *Liolaemus* occupied protected microhabitats at high altitude, whereas larger animals were able to utilize more exposed sites. However, Hertz and Huey (1981) found that smaller anolids occurred at higher altitude. Such results suggest that other factors, besides thermoregulation, may influence habitat selection at high altitude.

In contrast, *N. metallicus* from low altitude sites, while still occurring in structured habitats, occupied much more cryptic microhabitats. Low altitude animals were associated with wood basking surfaces, and tended to bask under complex vegetation in shade and lower to the ground. They also tended to occur very close to some form of retreat. This finding is in contrast to those from other studies of microhabitat selection by lizards in lowland areas, in which it has been reported that larger species are typically associated with more shaded areas. Conversely smaller species generally occupy warmer, more open microhabitats (Dent and Spellerberg, 1987; Scheibe, 1987; Paulissen, 1988). Nevertheless, at low altitudes *N. metallicus* probably occupies shaded microhabitats to reduce its risk of overheating. Bashey and Dunham (1997), who examined thermal constraints on the microhabitat preference of *Cophosaurus texanus*, reached a similar conclusion. At a low altitude warm site they found that only 20% of operative environmental temperatures were thermally acceptable during the mid day period. This compared to 70% for their high altitude colder site. They also reported similar habitat occupation to that observed for *N. metallicus*, with animals at low altitude generally occurring in areas of high cover and shade. Adolph (1990) also found preference for shade and cover in low altitude *Sceloporus* species, in comparison to high altitude populations.

Two other factors that may be responsible for observed patterns of basking site selection in *N. metallicus* are diet and predator avoidance. Diet is often one of the most important resources influencing an animal's distribution and community structure (Toft, 1985). However, *Niveoscincus* species are generally considered to be opportunistic active predators, eating predominantly insects and small amounts of plant material (Brown, 1991; Wapstra and Swain, 1996). Thus, as all animals occur in the more structured areas of available habitat and at each site insects were observed in great numbers, it appears unlikely that food quality or availability are in any way responsible for observed microhabitat occupation patterns.

Predator avoidance on the other hand may play a significant role in microhabitat occupation in this species. *Niveoscincus metallicus* is subject to both aerial predation from birds and ground predation, generally from snakes, especially *Drysdalia coronoides*, but also native and feral cats (*Dasyurus maculatus, Dasyurus viverrinus, Felis catus*). Predation pressure at both low altitude sites is probably stronger than at the Mt. Wellington site and may also exceed that at Clarence Lagoon. At low altitude sites large numbers of bird predators and small snakes are present; feral cats are also common at the Hobart site. Conversely at the Mt. Wellington site bird predators are rarely seen, although they are common at higher altitudes in Tasmania. Also the only ground predators observed here are snakes. Clarence Lagoon does have bird predators, but not in the numbers seen at the low altitude sites; snakes are also present here in large numbers.

Some lizards alter their anti-predator behaviour by selection of different distances to refugia (Rand, 1964; Bennet, 1980; Hertz *et al.*, 1982; Losos, 1988; Carrascal *et al.*, 1992). *Niveoscincus metallicus* displayed a tendency to select basking sites closer to refugia at lower altitude. This, combined with a tendency to bask under vegetation at lower altitude, would afford these animals substantial protection from visually based predators. Distance to refugia may therefore represent a measure of predation risk in this species. Carrascal *et al.* (1992) found that a high altitude lacertid, *Lacerta monticola*, displayed such behaviours, and concluded that distance to refugia was a measure of the trade-off between predator escape and thermal requirements. Variation in escape tactics resulting from changes in ecological pressures and/or physiological condition has been recorded in many studies (Hertz *et al.*, 1982; Crowley and Pietruszka, 1983; Snell *et al.*,

1988; Diaz, 1997). Indeed predator avoidance has been reported to represent a higher priority than thermoregulation in the nocturnal gecko *Oedura lesueurii* (Downes and Shine, 1998).

In order to truly understand habitat selection in any species of lizard, it is necessary to examine selection on a number of scales relevant to the animals studied (Smith, 1996; Rubio and Carrascal, 1994). Considering the proper scale is essential if results are to be generated that are important at the level of the organism, and not the investigator (Wiens, 1989; Levin, 1992; Smith, 1996). My investigation supports the conclusion that, across its altitudinal range, *N. metallicus* modifies the way it utilizes habitat, in ways that are consistent with the maintenance of some level of thermoregulatory precision (see Chapter 5). Thus this species is able to maintain a large distribution range by differential exploitation of appropriate microhabitats. However, a number of other factors, such as predator avoidance, are likely to be superimposed upon this behaviour.

CHAPTER FIVE

Thermal biology of *Niveoscincus metallicus*

5.1 Introduction

The maintenance of high and relatively constant body temperature through thermoregulatory mechanisms is a conspicuous aspect of lizard biology (Avery, 1982; Huey, 1982; Castilla and Bauwens, 1991; Bauwens *et al.*, 1996). Indeed, many species of lizard thermoregulate to maintain their body temperature between lower and upper threshold temperatures (e.g. Patterson and Davies, 1978; van Berkum *et al.*, 1986). Selection and regulation of body temperature is thought to be controlled by hypothalamic temperature receptors that are directly responsible for determining the thermal setpoints of an animal (Tosini and Avery, 1993). Setpoints represent the temperatures at which basking is initiated (lower setpoint) and ceases (upper setpoint) (Tosini and Avery, 1993). The resultant body temperatures experienced by an organism directly affect individual growth (Huey, 1982), defensive behaviour (Hertz *et al*, 1982; Losos, 1988) and reproduction (Andrews *et al.*, 1997). The physiological effects of body temperature can also affect energy intake (Avery *et al.*, 1982) and assimilation (Beaupre *et al.*, 1993). Similarly the thermal dependence of sprinting and stamina may influence an animal's ability to avoid predators (Bennett, 1980; Christian and Tracy, 1981; Hertz *et al.*, 1988; Mautz *et al.*, 1992). Existing data suggest that lizards are active at body temperatures that maximise performance. However, some studies have identified species or populations that exhibit serious locomotion impairment at field temperatures experienced in nature (Crowley, 1985; Huey and Bennett, 1987; Van Damme *et al.*, 1990). Consequently, populations living at the extremes of a species' range (e.g. high altitude) may be forced to undertake activities at suboptimal temperatures.

Lizards can respond in several ways to long term (e.g. seasonal, altitudinal) changes in thermal environment (Van Damme *et al.*, 1989; Spencer and Grimmond, 1994). Behavioural adjustments are generally thought to be the main compensatory mechanism controlling small scale spatial and temporal variation in a thermal environment (Hertz and

Huey, 1981; Avery, 1982; Van Damme *et al.*, 1987) and have been observed in *N. metallicus* (Chapter 4). However, behavioural adaptations may be inadequate to compensate for differing thermal loads, or they may be too costly in terms of time and energy expenditure (Huey and Slatkin, 1976). Under such conditions a lizard may display some level of physiological acclimatization to varying environmental conditions (Van Damme *et al.*, 1989). Consequently, physiological acclimatization, acting more slowly than behavioural adjustments, may extend the range of suitable thermal environments, both seasonally and geographically (Veron and Heatwole, 1970; Crowley, 1985).

The extent to which lizard populations or congeneric species are able to utilize genetic adaptations of thermal physiology is not fully resolved. If short-term behavioural adjustments or acclimatization results in the maintenance of body temperatures that are conducive to reduced physiological performance, natural selection should favor a shift in the physiological optimum temperatures and/or the tolerance zone in order to restore maximum performance (Hertz *et al.*, 1983; Van Damme *et al.*, 1989). Although some workers suggest that thermal physiology is responsive to directional selection and thus reflects genetic adaptation, others feel that because thermoregulatory adjustments effectively minimize geographic variability in body temperature, thermal physiology is evolutionarily conservative (Gillis, 1991). These theories are generally referred to as the "labile" view and the "static" view (see Hertz *et al.*, 1983). Data presented in support of either view are generally indirect measures of the thermal sensitivity of performance. Such measures include critical thermal limits and body temperatures selected in the laboratory and field (van Berkum, 1986). Direct measures of physiological performance (e.g. sprint speed) tend to support the static view (Hertz *et al.*, 1983), but see van Berkum (1986) for an exception.

Ectotherms inhabiting large altitudinal ranges provide an excellent opportunity to study various aspects of thermal adaptation. Populations at the edge of a species' climatic or distributional range, such as *N. metallicus* at the high altitude sites in this study, are often forced to maintain activity under temperature conditions that are suboptimal. Kik (1998) and Kabat (1999) have both shown that *Niveoscincus microlepidotus*, a sister species of *N. metallicus*, lowers its thermal setpoints and preferred body temperature during pregnancy. The possibility therefore exists that *N. metallicus* is able to alter aspects of its thermal biology in response to not just pregnancy, but also geography. *Niveoscincus metallicus* is

able to utilize behavioural modifications to adapt to thermal variations between high and low altitude sites (Chapter 4). This chapter examines the importance of acclimation and genetic adaptation in allowing *N. metallicus* to maintain the large altitudinal range it displays. The study integrates field and laboratory data on thermal physiology and performance. This allows examination of the degree of coincidence between the body temperatures of active lizards in their natural environment and the physiological responses exhibited in the laboratory. Five specific questions were examined in this study.

1) Does *N. metallicus* maintain the same preferred body temperature (T_b) across its altitudinal range and is there any relationship between T_b maintained in the field, and ground temperature (T_s) or air temperature (T_a)?
2) Is *N. metallicus* able to maintain eccritic temperatures selected in the laboratory, at each field site, and is there evidence that aspects of thermal behaviour in the laboratory differ between populations?
3) Are population differences in thermal performance or thermal limits evident in *N. metallicus*?
4) To what extent does physiological acclimation allow *N. metallicus* to adapt to its thermal environment?
5) Is there evidence that genetic adaptation of thermal performance and/or thermal limits has occurred in populations of *N. metallicus*?

5. 2 Materials and Methods

5.2.1 Field temperatures

Observations were made at the four previously described field sites (Chapter 2) during the summers of 1998 and 1999. Animals were captured only on clear days with a maximum air temperature of not less than 19°C and not more than 25°C. Data were recorded by starting from a randomly determined location and moving slowly back and forth across the site to ensure coverage of the entire range of microhabitats available. Collections commenced at 7.00am and continued until animals submerged. As many lizards as possible were captured; however, only pregnant females were examined at each site to minimize variation induced by differences in the timing of reproductive events (Chapter 1). For each lizard caught I recorded the time of day, sex, air temperature (shaded air temp

15 cm above substrate = T_a), shaded substrate temperature (T_s), and cloacal temperature (T_b). A small thermister probe was used to measure temperatures to within ±0.1°C. Cloacal temperatures were recorded within 15 seconds of capture to avoid any effects caused by handling of lizards for extended periods (Marquet *et al.*, 1989).

5.2.2 Eccritic temperatures, basking setpoints and thermal behaviour in the laboratory

A large open topped terrarium (120 cm x 120 cm x 30 cm) was used as a test area. It was subdivided into 8 separate sections (55 cm x 30 cm) by wooden partitions. Each section was given a number (1 to 8). A 2 to 3 cm layer of sand was provided as a substrate to animals and each section was provided with a terracotta bowl, upturned to act as a basking surface. A heat source (25 watt light bulb) was fixed approximately 6 cm above the basking surface on specially designed racks. This apparatus provided a temperature gradient of below 15°C to above 35°C in each section. Animals were not fed for 24 hour prior to a trial or during the trial; however, water was provided *ad libitum.*

Body temperatures were recorded at 2 minute intervals over the course of the day (9am-6pm. Small temperature probes (0.5 mm diameter) were inserted into the cloaca and securely held in place with a narrow strip of adhesive electrician's tape. Animals prepared in this fashion were distributed between the eight test chambers. Each probe was connected to a data logger by a 1.5 m lead. This lead allowed free movement of an animal around its experimental enclosure and did not appear to interfere with shuttling heliothermy (Figure 5.1). Probes were connected to a data logger, which in turn downloaded results directly to a Macintosh™ computer. Animals were familiarized with the test area, with a cloacal probe in place, approximately half an hour before the commencement of each trial. Randomly selected adult animals were tested from each of the field sites. Both females and males were examined, and animals were studied during both spring and autumn. Approximately eight animals were used in each treatment group.

Prior to each trial lizards were marked with a non-toxic white marker for identification. Animals were measured (SVL) and weighed (± 0.1 mg) and reproductive condition of females determined by palpation. They were then transferred to test compartments. For

each animal the overall mean temperature (average body temperature over the entire activity period: T_{ave}), mean upper and lower basking setpoints (average body temperature where basking initiated and terminated), maximum and minimum temperatures (highest and lowest body temperature recorded), time spent at high temperatures (>30°C, >35°C) and time at low temperatures (<20°C) were recorded. Body temperatures were recorded over a nine hour period (Figure 5.1).

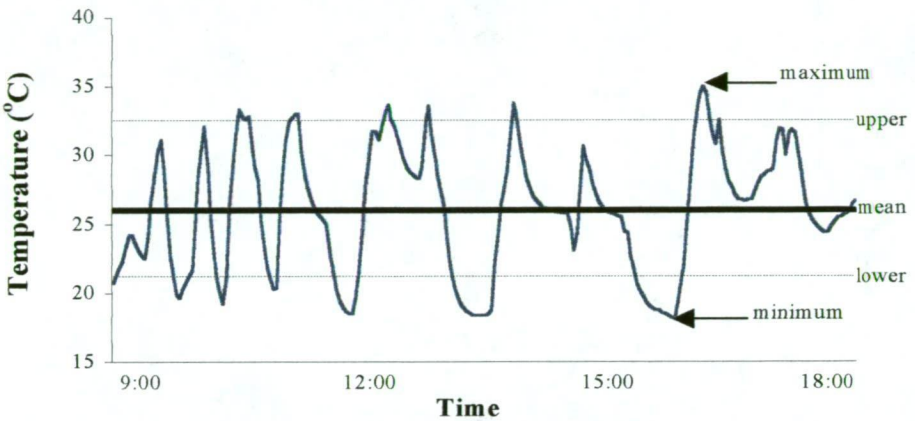

Figure 5.1 Representative body temperature profile recorded for a male *N. metallicus* from Orford during autumn. Lines identify the setpoints calculated for this animal (upper and lower) as well as the T_{ave}. The arrows identify the maximum and minimum body temperatures recorded for the animal.

5.2.3 Critical thermal limits

The upper and lower temperatures at which a lizard loses it ability to right itself are commonly used as an indicator for the loss of muscle co-ordination and the ability to move. This measure is considered to be ecologically relevant, because if a lizard's body temperature goes beyond this point death will occur (Bennett and John-Alder, 1986). Non-lethal loss of righting response was measured according to the procedures described by Bennett and John-Alder (1986).

Measurements of critical thermal maximum (CTmax) were obtained by placing lizards in a Qualtex thermostat controlled oven at 30°C for 30 minutes. Following equilibration a small thermister probe was placed in the animal's cloaca to record subsequent measures of T_b. The temperature was then raised at a rate of 1°C / 2 min until the lizard appeared uncoordinated. At this point the animal was placed on its back to determine whether righting ability was lost. As soon as this occurred T_b was recorded. The lizard was then

transferred to a container immersed in cold water; all animals recovered successfully from this procedure.

Critical thermal minimum (CTmin) was determined in comparable fashion, except that animals were first equilibrated to 15°C and were then cooled on a sheet of aluminum foil laid on crushed ice. T_b decreased at a rate of about 1°C / min. As soon as righting ability was lost T_b was recorded and the animal was transferred to a container immersed in a warm water bath. Again all animals recovered successfully. No animal was used more than once for any measure of CTmax or CTmin.

Critical thermal limits were obtained for adults of both sexes from all field sites in the late spring of 1998 and autumn of 1999. All estimates of CTmax and CTmin were determined within 3 days of arrival at the laboratory. A third group of animals was also captured in spring and held in the laboratory for 3 months to examine laboratory acclimation. Critical thermal limits of newborn young were also determined to investigate potential genetic adaptation. For this study females from all sites were held in the laboratory in standard conditions from early pregnancy (embryonic stage 35) until they gave birth. Following birth young were weighed and measured. They were not sexed as this can be damaging to very small lizards and is unreliable in *N. metallicus*. Young were divided into groups of six (SVL >20 mm and <22 mm). One of these young was killed (frozen at –20°C) and allowed to thaw to room temperature with the remaining young. A small thermister probe was inserted into its abdomen and all animals in the group (including the dead control subject) were placed into equilibration conditions as described above. After 30 minutes young were transferred to test conditions and CTmax and CTmin determined. This was recorded as the temperature of the dead control subject, held under identical conditions to test subjects, when the test animals lost righting ability.

5.2.4 Thermal sensitivity of sprint speed and willingness to run

There is a significant relationship between sprint speed and temperature in reptiles (Huey and Stevenson, 1979; Huey, 1982; Van Damme *et al.*, 1989). Sprinting is maximized as temperature approaches an optimum, after which point performance begins to decline. Melville (1998) has reported that *N. metallicus* primarily uses sprinting as an escape method in natural habitats. Lizards were collected in autumn, following parturition, from

the four study sites. Ten adults of each sex were used from each site and sprint curves were constructed over a range of temperatures (17, 20, 23, 26, 29, and 32°C). Animals were run at these temperatures in randomised order. A heated racetrack and a lizard-heating device were designed for a previous study at the university, and were available for this work. The track was 2.5 m long with a metal bottom, lined with sandpaper to allow traction. It sat on a box containing 3 x 120-watt spotlights. A temperature probe linked to a control box switched these lights off and on and allowed the track to be rapidly heated and maintained to within 1°C of a required temperature. In all experiments the track temperature was set to 1°C above the test temperature. Three light beams at 50 cm intervals, linked to a Macintosh™ computer, allowed sprint time over 50 cm to be determined. Consequently, each trial gave a maximum of 2 estimates of sprint speed. For each run, I also recorded the number of times the individual attempted to stop running over the 1 m of track. This was used as an indication of an individual's willingness to use sprinting as an escape tactic at the temperature tested.

Animals were warmed in the lizard heating device (box immersed in a water bath) for 30 minutes prior to each sprint trial. The water temperature was controlled by a heater, thermostat (±1°C) and small propeller. This method of heating is an improvement on methods where heating is achieved from one surface, as temperature is less variable (Melville, 1998). Following this, lizards were chased down the track with a small brush. Care was taken never to break a light beam before the lizard. Each animal was raced three times down the track; however, only the fastest time over 50 cm was recorded as the maximum sprint speed and only the run where the animal stopped the fewest times was recorded as the stopping frequency. Only one temperature was tested each day.

5.2.5 Data analysis

All data were analysed using SAS System for Windows©v6.12. Mean T_b, T_s and T_a were examined using two-way ANOVA with site and sex as factors. Homoscedacity of variances was investigated by visual inspection of plots of group standard deviations versus group means. Normality of the data was evaluated through the inspection of a normal probability plot. Significant interactions were further examined using Tukey's studentized range tests. The relationships between T_b and T_s, and T_b and T_a were assessed

using factorial Analysis of Covariance, with T_a and T_b acting at the co-variates and site and sex acting as factors. The assumption that each treatment had identical group regression slopes was tested by assessing the significance of the treatment*co-variate interactions. If the treatment*co-variate interactions proved to be significant, I used regression analysis followed by multiple comparisons of slopes ANOVA (*post-hoc* test) to determine which of the regression equations displayed slope variation. Assumptions of regression analyses were examined using plots of residuals against predicted values, normal probability plots and plots of Cook's D estimate against leverage. Assuming the ANCOVA assumption of equal slopes was meet, the analysis continued. Linearity of the relationship between the dependent variable and the co-variate was evaluated by examining a plot of the residuals versus the co-variate and assessing the normality of residuals. Finally, the independence of residual variances and response variables was assessed by examining a plot of residuals against predicted values and comparing the variance of residuals among groups. I also examined the relative contributions of T_a and T_s on the dependent variable T_b, using simple multiple regression. This analysis was conducted individually on males and females from each site.

Eccritic temperatures, basking setpoints and thermal behaviour in the laboratory were examined using two-way ANOVAs. Factors examined were time of collection (spring or autumn) and site of collection. Sexes were analysed separately to prevent confusion in data interpretation. Homoscedacity of variances and normality of the data were evaluated as described above. *Post-hoc* tests were employed where appropriate.

Two-way ANOVAs were used to determine whether CTmax and CTmin differed between sites and collection periods. Analyses were performed separately for both sexes. Homoscedacity of variances and normality of data were assessed in the manner described above. Significant interactions were further examined using Tukey's studentized range tests. Differences between CTmax and CTmin for newborn young from each site was assessed using one-way ANOVAs. Homoscedacity of variances and normality of data were assessed in the manner described above. Again significant interactions were further examined using Tukey's studentized range tests.

Thermal sensitivity of sprint speed was analysed initially using a factorial repeated measures ANCOVA design, with SVL and weight as the co-variates and study animal as

the repeated factor. The importance of the co-variate was assessed; however, in each case it was not significant. I therefore continued the analysis using factorial repeated measures ANOVAs for both the sprint speed and stopping data. The factors examined were study site (normal factor) and individual animal (repeated measure factor). Sexes were again analysed individually to aid in interpretation of results. The assumptions of group variances and normality were assessed by visual inspection of plots of group standard deviations versus group means and estimated versus predicted residual values, respectively. The traditional method of analysis of sprint speed curves (analysis of optimal temperatures and indices of performance) was not used here as it failed to effectively describe differences between populations.

5. 3 Results

5.3.1 Field temperatures

Mean body temperature (T_b), substrate temperatures (T_s) and air temperatures (T_a) for adult males and females from all study sites are presented in Table 5.1. In all cases, two-way ANOVAs revealed that site alone varied between groups ((T_b)$F_{3,318} = 4.10$, $P = 0.0071$; (T_s) $F_{3,318} = 8.20$, $P = 0.0001$; (T_a) $F_{3,318} = 7.73$, $P = 0.0001$). In no case was a significant sex or interaction effect recorded. *Post-hoc* examinations revealed that for T_b, Clarence lagoon animals had significantly higher values than animals from either Hobart or Orford ($P < 0.05$). No other T_b comparisons were significant. The T_s results revealed that Mt Wellington values were lower than for either low altitude site, while those from Clarence Lagoon were lower than those obtained from animals at Orford ($P < 0.05$). The result for T_a produced a pattern that was different again, with Orford values being higher than those recorded from animals at Mt. Wellington and Hobart. Clarence Lagoon values were also significantly higher than those recorded at Mt. Wellington.

Table 5.1 Mean body temp (T_b), substrate temperatures (T_s) and air temperatures (T_a) for adult male and female *N. metallicus* from all field sites sampled. Values are means ± s.e.

Site	Sex	T_b ± s.e.	T_s ± s.e.	T_a ± s.e.
Clarence Lagoon	Female	30.6 ± 0.40	26.6 ± 0.65	20.8 ± 0.57
	Male	30.8 ± 0.41	26.1 ± 0.68	20.0 ± 0.60
Mt. Wellington	Female	29.7 ± 0.38	25.8 ± 0.56	18.8 ± 0.46
	Male	29.8 ± 0.40	25.6 ± 0.51	18.1 ± 0.57
Orford	Female	29.3 ± 0.45	29.2 ± 0.64	21.0 ± 0.46
	Male	29.8 ± 0.35	28.1 ± 0.57	20.8 ± 0.38
Hobart	Female	29.2 ± 0.31	27.7 ± 0.55	19.6 ± 0.49
	Male	30.0 ± 0.38	26.9 ± 0.63	19.1 ± 0.77

The results of the factorial ANCOVA examining T_b after adjusting for the continuous independent variable T_s indicated that the sum of the interaction effects of the co-variate with the treatment effect was not significant ($F_{calc(7,311)} = 0.389$, $P > 0.75$). Thus, it can be concluded that there is homogeneity of slopes among all groups (common slope = 0.298). The ANCOVA proper indicated significant effects of site ($F = 10.57$, $P < 0.0001$), sex ($F = 6.51$, $P = 0.0112$) and of course the co-variate T_s ($F = 90.81$, $P < 0.0001$); however, no interaction effect between site and sex was observed ($F = 0.53$, $P = 0.66$). Two general trends are evident from these data (Figure 5.2). The first is that, irrespective of site, females display significantly lower least squares (adjusted for T_s) means than males. Thus, for any given T_s the corresponding T_b of a female will be slightly lower. This trend is especially strong at the low altitude sites. The second trend is that animals from the two high altitude sites (Clarence Lagoon and Mt Wellington) appear to have higher least squares mean temperatures (and therefore y-intercepts) than do low altitude animals (Hobart and Orford). Thus at any T_s an animal from a high altitude site will have a corresponding higher T_b than an animal from a low altitude site. This is most evident for female animals.

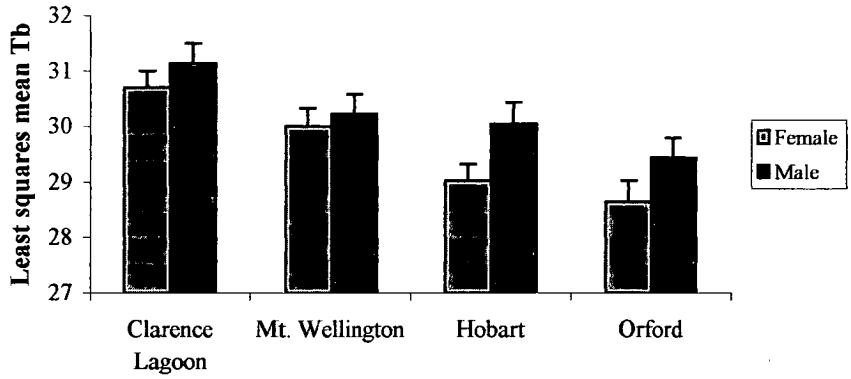

Figure5.2 Mean body temperatures of male and female *N. metallicus* from the high altitude (Clarence Lagoon and Mt. Wellington) and low altitude (Hobart and Orford) study sites. All values represent least squares means adjusted for T_s (± s.e.).

The second factorial ANCOVA, examining T_b after adjusting for the continuous independent variable T_a, indicated that the sum of the interaction effects of the co-variate with the treatment effect was significant ($F_{calc(7,311)} = 2.735$, $0.01 > P > 0.005$) indicating that there was not homogeneity of slopes among all groups. Consequently an ANCOVA could not be conducted. Thus, I continued the analysis using least square linear regression. Significant relationships between T_b and T_a were evident for each site/sex combination except for Clarence Lagoon males and Hobart males (Table 5.2).

Table 5.2 Regression equations ($y = a + bx$) relating body temperature (T_b) to air temperature (T_a) in males and females from all four study sites.

Site	Sex	Slope (b)	Intercept (a)	R^2	ANOVA Df	*F*	*P*
Clarence Lagoon	**F**	0.265	25.08	0.144	1,49	8.24	0.006
	M	0.146	28.03	0.047	1,36	1.79	0.19
Mt. Wellington	**F**	0.553	19.26	0.454	1,42	34.91	0.0001
	M	0.281	24.73	0.167	1,37	7.42	0.0098
Hobart	**F**	0.305	23.82	0.230	1,50	14.94	0.0003
	M	0.116	27.82	0.054	1,30	1.72	0.19
Orford	**F**	0.635	15.93	0.410	1,30	20.77	0.0001
	M	0.494	19.52	0.293	1,37	15.34	0.0004

A multiple comparison of slopes ANOVA (*post-hoc* test) demonstrated that there were significant differences between the slopes of site/sex groups ($F_{12,315} = 7.62$, $P < 0.0001$). What becomes apparent in this case is that the slope of the relationship in females is steeper and the y-intercept lower than in males, irrespective of site. Thus males appear to show greater independence of T_b from T_a. However, superimposed on this are site variations. Unlike the T_b / T_s analysis, which grouped sites according to altitude, this analysis groups sites according to the complexity of the habitat occupied. Clarence Lagoon and Hobart *N. metallicus* (complex sites (Chapter 4)) display lower slopes and higher y-intercepts than animals from open habitat sites (Orford and Mt. Wellington) (Figure 5.3).

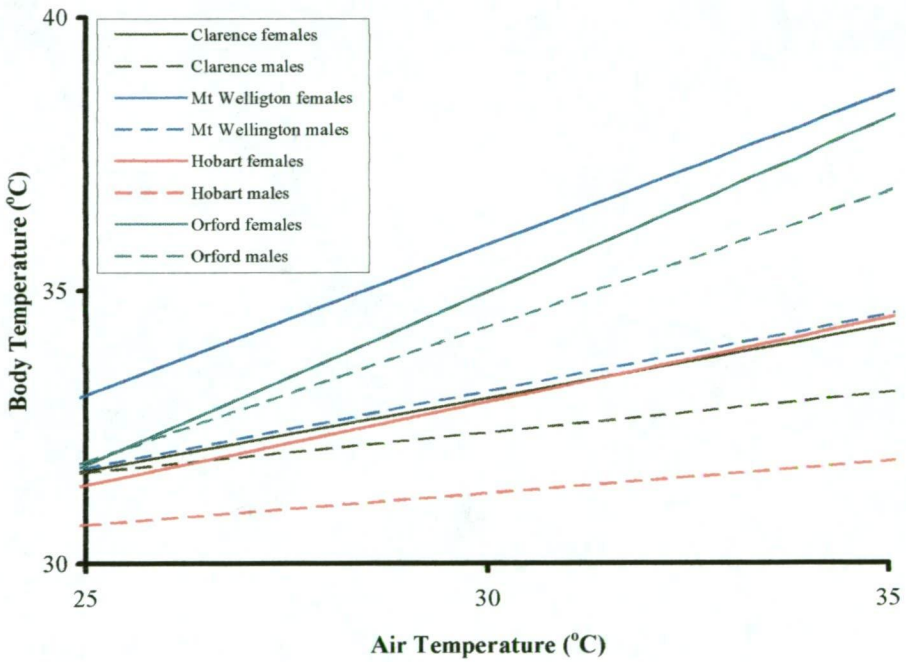

Figure 5.3 Regression slopes for the relationship between body and air temperatures in adult males and females from all four study sites. Note that the y-intercept values are not shown and that lines intersect prior to the y-intercept. See Table 5.2 for regression equations.

The results of the simple multiple regression examining the relative contribution of the independent variables T_a and T_s to the dependent variable T_b are shown in Table 5.3. In all cases the global null hypothesis of the models, that all slopes equal zero, was rejected ($P < 0.05$, results listed below). In all regressions from the sites with complex habitat structure (Clarence Lagoon and Hobart) correlations between T_a and T_b were lower than those between T_s and T_b. The opposite was generally true of open sites (Orford and Mt Wellington); however, Mt Wellington males showed a pattern similar to that observed at

closed sites. In only one case was the correlation between independent variables greater than 0.7 (Clarence Lagoon females).

Table 5.3 Results of simple multiple regressions examining the relative contributions of T_a and T_s in explaining variation in T_b at each of the four study sites. Male and female animals were analyzed separately. Data presented are overall model results, significance test of independent variables, the semi-partial correlation indicating the unique contribution of an independent variable to the dependent variable, and the total R-squared value of the model.

Site	Sex	Variable	DF of Model	*F* of Model	*P* of Model	*P* of Variable	Squared semi-partial correlation	R-squared value of Model
Hobart	M	T_s	2,29	3.50	0.044	**0.033**	0.140	0.194
Hobart	M	T_a				0.710	0.004	
Hobart	F	T_s	2,49	16.45	0.001	**0.001**	0.172	0.402
Hobart	F	T_a				**0.009**	0.092	
Orford	M	T_s	2,36	9.12	0.001	0.135	0.043	0.336
Orford	M	T_a				**0.002**	0.203	
Orford	F	T_s	2,29	10.95	0.001	0.307	0.021	0.430
Orford	F	T_a				**0.001**	0.246	
Clarence	M	T_s	2,35	3.32	0.048	**0.038**	0.112	0.160
Clarence	M	T_a				0.629	0.006	
Clarence	F	T_s	2,48	7.54	0.001	**0.018**	0.095	0.239
Clarence	F	T_a				0.940	0.001	
Wellington	M	T_s	2,36	7.47	0.002	**0.016**	0.126	0.293
Wellington	M	T_a				0.190	0.035	
Wellington	F	T_s	2,41	25.14	0.001	**0.005**	0.097	0.551
Wellington	F	T_a				**0.001**	0.253	

5.3.2 Eccritic temperatures, basking setpoints and thermal behaviour in the laboratory

Eccritic temperatures, basking setpoints and thermal behaviour in the laboratory were examined using two-way ANOVAs. The results of these analyses are shown in Tables 5.4, 5.5, 5.6 and 5.7.

Table 5.4 Mean upper and lower set points (°C ± s.e.) for animals collected in spring and autumn from the four study sites. The results of the factorial ANOVA of these data are also presented.

Variable	Group	Orford	Hobart	Clarence	Wellington	Season		Site		Interaction	
	sex/season					*F*(df)	*P*	*F*(df)	*P*	*F*(df)	*P*
Upper setpoints	M/spring	32.6 ± 0.49	32.9 ± 0.56	32.9 ± 0.38	32.9 ± 0.63	0.12 (1,45)	0.734	0.22 (3,45)	0.884	0.15 (3,45)	0.927
	M/autumn	32.8 ± 0.49	33.1 ± 0.59	32.6 ± 0.56	33.3 ± 0.39						
	F/spring	32.6 ± 0.34	31.5 ± 1.08	32.6 ± 0.47	31.5 ± 0.75	4.96 (1,49)	**0.031**	2.29 (3,49)	0.090	0.04 (3,49)	0.991
	F/autumn	33.9 ± 0.90	32.6 ± 0.49	33.8 ± 0.21	32.3 ± 0.41						
Lower setpoints	M/spring	21.3 ± 0.67	19.8 ± 0.19	22.8 ± 0.46	22.2 ± 0.40	0.57 (1,45)	0.455	3.70 (3,45)	**0.018**	3.91 (3,45)	**0.014**
	M/autumn	21.3 ± 0.36	21.2 ± 0.72	21.1 ± 0.28	21.4 ± 0.51						
	F/spring	22.2 ± 0.49	24.2 ± 1.25	22.7 ± 0.32	22.4 ± 0.57	14.79 (1,49)	**0.0003**	0.57 (3,49)	0.639	1.14 (3,49)	0.341
	F/autumn	20.7 ± 0.37	20.6 ± 0.22	21.2 ± 0.65	21.0 ± 0.16						

Table 5.5 Mean and maximum body temperatures (°C ± s.e.) for animals collected in spring and autumn from the four study sites. The results of the factorial ANOVA of these data are also presented.

Variable	Group	Orford	Hobart	Clarence	Wellington	Season		Site		Interaction	
	sex/season					*F*(df)	*P*	*F*(df)	*P*	*F*(df)	*P*
T_{ave} (°C)	M/spring	25.3 ± 0.30	24.8 ± 0.32	26.2 ± 0.52	25.9 ± 0.48	1.11 (1,45)	0.298	0.39 (3,45)	0.761	0.71 (3,45)	0.553
	M/autumn	25.2 ± 0.57	25.1 ± 0.72	24.8 ± 0.49	25.3 ± 1.00						
	F/spring	27.0 ± 0.46	26.8 ± 0.42	25.9 ± 0.29	25.0 ± 0.46	3.13 (1,49)	0.083	1.39 (3,49)	0.256	0.87 (3,49)	0.464
	F/autumn	25.8 ± 1.00	25.3 ± 0.89	25.3 ± 0.58	25.3 ± 0.58						
Max temp (°C)	M/spring	34.7 ± 0.59	35.2 ± 0.58	35.6 ± 0.16	35.0 ± 0.92	3.06 (1,45)	0.087	1.14 (3,45)	0.342	0.59 (3,45)	0.627
	M/autumn	35.1 ± 0.50	35.6 ± 0.62	35.9 ± 0.50	36.6 ± 0.31						
	F/spring	35.5 ± 0.75	34.1 ± 0.42	34.7 ± 0.51	34.3 ± 1.31	6.07 (1,49)	**0.017**	1.29 (3,49)	0.287	0.42(3,49)	0.740
	F/autumn	36.2 ± 0.88	35.8 ± 0.43	36.6 ± 0.29	35.0 ± 0.71						

Table 5.6 Minimum body temperatures (°C ± s.e.) and proportion of time spent under 20 °C (%) for animals collected in spring and autumn from the four study sites. The results of the factorial ANOVA of these data are also presented.

Variable	Group	Orford	Hobart	Clarence	Wellington	Season		Site		Interaction	
	sex/season					*F*(df)	*P*	*F*(df)	*P*	*F*(df)	*P*
Min temp (°C)	M/spring	17.7 ± 0.28	17.4 ± 0.09	18.9 ± 0.39	18.5 ± 0.54	11.74 (1,45)	**0.001**	1.73 (3,45)	0.174	4.40 (3,45)	**0.008**
	M/autumn	17.7 ± 0.18	17.4 ± 0.42	17.2 ± 0.10	17.3 ± 0.18						
	F/spring	18.8 ± 0.45	19.3 ± 0.45	18.6 ± 0.24	18.1 ± 0.16	13.97 (1,49)	**0.0005**	1.05 (3,49)	0.378	0.48 (3,49)	0.699
	F/autumn	16.9 ± 0.88	17.7 ± 0.33	17.6 ± 0.70	17.3 ± 0.30						
Under 20°C	M/spring	14.9 ± 1.71	25.1 ± 2.45	9.4 ± 2.61	9.6 ± 3.94	7.84 (1,45)	**0.008**	1.36 (3,45)	0.268	2.11 (3,49)	0.113
	M/autumn	23.5 ± 4.33	21.3 ± 4.46	20.9 ± 1.92	26.9 ± 7.02						
	F/spring	6.3 ± 2.10	5.8 ± 2.76	11.1 ± 2.16	15.1 ± 1.82	13.93 (1,49)	**0.0005**	0.91 (3,49)	0.443	1.23 (3,49)	0.308
	F/autumn	17.2 ± 6.80	20.6 ± 4.74	23.2 ± 5.55	16.1 ± 1.47						

Table 5.7 Proportion of time spent (%) above 35 °C and 30 °C (± s.e.) for animals collected in spring and autumn from the four study sites. The results of the factorial ANOVA of these data are also presented.

Variable	Group	Orford	Hobart	Clarence	Wellington	Season		Site		Interaction	
	sex/season					*F*(df)	*P*	*F*(df)	*P*	*F*(df)	*P*
Above 35°C	M/spring	0.9 ± 0.56	0.5 ± 0.28	1.4 ± 0.47	1.3 ± 0.78	5.22 (1,45)	**0.027**	0.92 (3,45)	0.438	0.38 (3,45)	0.768
	M/autumn	1.2 ± 0.39	2.3 ± 0.84	2.5 ± 1.01	2.9 ± 0.92						
	F/spring	0.5 ± 0.20	0.5 ± 0.37	0.7 ± 0.39	1.3 ± 0.93	9.88 (1,49)	**0.003**	1.91 (3,49)	0.141	2.43 (3,49)	0.077
	F/autumn	6.6 ± 4.34	1.6 ± 0.40	2.4 ± 0.39	2.1 ± 1.18						
Above 30°C	M/spring	15.2 ± 1.99	13.6 ± 1.84	25.0 ± 3.92	20.6 ± 5.15	0.79 (1,45)	0.378	0.99 (3,45)	0.406	0.80 (3,45)	0.503
	M/autumn	21.0 ± 4.52	19.5 ± 4.04	19.8 ± 3.96	25.6 ± 6.21						
	F/spring	25.1 ± 4.73	20.7 ± 3.50	21.1 ± 2.88	21.0 ± 2.30	0.13 (1,49)	0.720	0.09 (3,49)	0.964	0.23 (3,49)	0.875
	F/autumn	22.2 ± 10.10	25.7 ± 8.12	24.7 ± 2.40	20.7 ± 5.73						

The most obvious result from the above analyses is that T_{ave} was not influenced by either collection date or site. Collection site alone did not appear to significantly affect any aspect of thermal behaviour in the laboratory. Animals of both sexes from high and low altitude populations displayed similar upper setpoints, T_{ave}, maximum body temperatures, minimum body temperatures, time spent below 20°C, time spent above 30°C and time spent above 35°C. Females also showed no site effect for lower setpoints, but males did display a site effect for this factor. However, the effect was influenced by date of capture. A significant difference was observed between Hobart and Clarence Lagoon males collected during spring. This may reflect a general difference between high and low altitude sites in relation to this variable, as both high altitude populations appear to have slightly higher lower setpoints (T_{bask}) at this time of year.

In contrast to site of collection, date of collection appears to significantly influence aspects of thermoregulation, other than T_{ave}, in the laboratory. This was especially evident for female animals. Upper and lower setpoints, maximum and minimum body temperatures, time spent above 35°C and time spent below 20°C were all significantly influenced by date of capture. All data supported the conclusion that females were controlling their body temperature more precisely during spring, when all of them were pregnant. Male animals also showed a significant effect in relation to date of collection for the time they spent with T_b below 20 °C and time spent above 35 °C. Males appeared to follow a similar pattern to females, spending less time at extreme temperatures in spring. They also showed a significant interaction effect between collection date and site for minimum body temperature. A similar pattern to that described for the interaction effect for lower setpoints was evident. Male *N. metallicus* from Clarence Lagoon, collected in spring, displayed significantly higher minimum body temperatures than animals from all autumn collections and from Hobart animals in spring ($P < 0.05$). Values approached, but did not reach, significance in the comparison with animals collected at Orford in spring.

5.3.3 Critical thermal limits

The upper and lower critical thermal limits of adult *N. metallicus*, from all four locations and for all experimental periods, are presented in Figures 5.4, 5.5, 5.6 and 5.7.

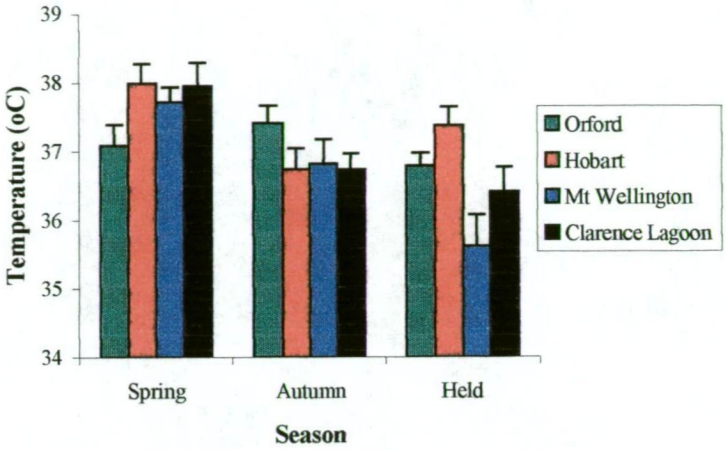

Figure 5.4 Upper critical thermal limits (CTmax) of male *N. metallicus*, from the four collection sites, captured and tested in spring and autumn, and captures in spring then held in the laboratory for 4 months. Data are means ± s.e.

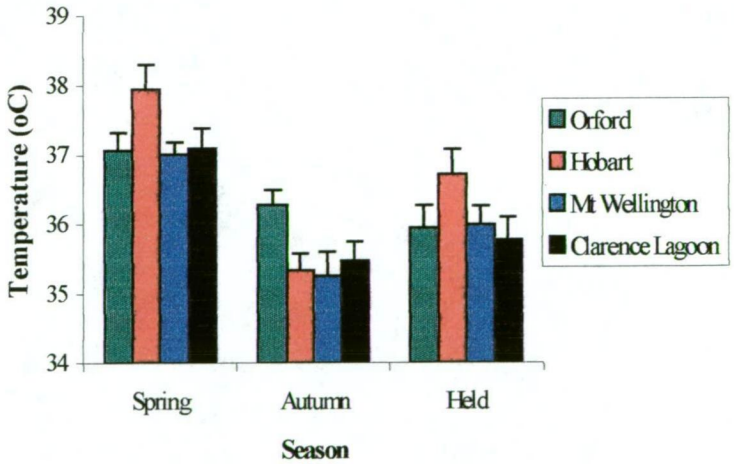

Figure 5.5 Upper critical thermal limits (CTmax) of female *N. metallicus*, from the four collection sites, captured and tested in spring and autumn, and captures in spring then held in the laboratory for 4 months (i.e. throughout gestation). Data are means ± s.e.

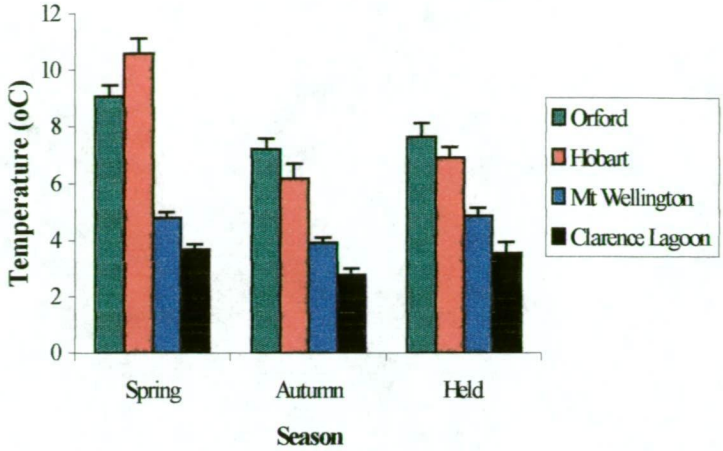

Figure 5.6 Lower critical thermal limits (CTmin) of male *N. metallicus*, from the four collection sites, captured and tested in spring and autumn, and captures in spring then held in the laboratory for 4 months. Data are means ± s.e.

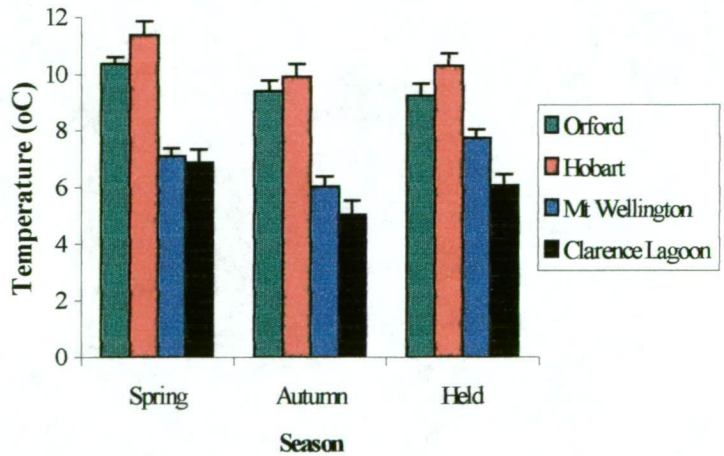

Figure 5.7 Lower critical thermal limits (CTmin) of female *N. metallicus*, from the four collection sites, captured and tested in spring and autumn, and captures in spring then held in the laboratory for 4 months (i.e. throughout gestation). Data are means ± s.e.

Each of the above graphs was interpreted using a two-way ANOVA with site and collection period as factors. Results for CTmax for males (Figure 5.3) indicate that collection period and site of capture interact significantly ($F_{6,102} = 3.14, P = 0.0072$). *Post-hoc* tests of interest indicate that animals from Mt Wellington and Clarence Lagoon (high altitude sites) had significantly lower CTmax values for animals captured in spring and

held for 4 months than were evident in animals captured and examined in spring ($P < 0.05$). High altitude animals captured and studied in autumn were not significantly different from either of the other collection groups. No site effects were evident within any collection period.

The comparable results for CTmax for females (Figure 5.4) also indicate that collection period and site of capture interact significantly ($F_{6,100} = 2.20$, $P = 0.0486$). Again no site effects were evident within any collection period. Of interest are the significant differences observed between animals from Hobart, Mt Wellington and Clarence Lagoon, for collections captured and studied during spring and autumn ($P < 0.05$). Thus for these three sites, CTmax values dropped over this period. However, while CTmax values appeared lower for a female captured in spring and held until autumn, they were not significantly different from either the spring or autumn data.

A significant interaction effect was also observed in males for CTmin data (Figure 5.5) ($F_{6,102} = 7.14$, $P = 0.0001$). A general trend observed across all collection periods was the lower CTmin of high altitude animals ($P < 0.05$), and in all cases low altitude and high altitude groupings were formed ($P > 0.05$). Also of interest in this case is the greater number of between collection differences observed within a site. Orford animals displayed a significant difference between spring and autumn collections. Hobart animals collected in spring displayed significantly greater CTmin values than either those collected in autumn or those held from spring in the laboratory. The latter two collections were not significantly different. No differences were evident for high altitude sites between collection periods.

Finally, CTmin values for females from all populations were examined (Figure 5.6). In this case collection period ($F_{2,99} = 10.87$, $P = 0.0001$) and collection site ($F_{3,99} = 82.52$, $P = 0.0001$) were found to affect CTmin. However, there was no interaction effect ($F_{6,99} = 1.43$, $P = 0.21$). What is apparent from a *post-hoc* examination of these data is that animals collected in autumn and those collected in spring and held until autumn did not differ. Spring collected animals, however, tend to display high CTmin values ($P < 0.05$). A comparison of differences between sites indicated that all sites were different at the 0.05 level. However, the greatest differences were again observed between high and low altitude groupings.

The final examination of CTmax and CTmin values was made for newborn animals born in the laboratory to mothers held from early gestation. The results for these animals are displayed in Table 5.8.

Table 5.8 CTmax and CTmin values for newborn *N. metallicus* born in the laboratory from mothers collected early in gestation (before embryonic stage 35) from the four field sites. Values displayed are means (±s.e..).

Site	Ctmax ± s.e. (N)	CTmin ± s.e. (N)
Orford	41.2 ± 0.13 (19)	9.9 ± 0.43 (19)
Hobart	41.8 ± 0.19 (20)	9.3 ± 0.41 (20)
Clarence Lagoon	40.4 ± 0.06 (18)	3.7 ± 0.22 (18)
Mt Wellington	40.4 ± 0.08 (20)	4.2 ± 0.21 (19)

One way ANOVAs revealed that both CTmax ($F_{3,73} = 28.63$, $P = 0.0001$) and CTmin ($F_{3,72} = 94.10$, $P = 0.0001$) differed between field sites. CTmax values failed to differ significantly between only the two high altitude sites ($P > 0.05$); they were higher for both low altitude populations. CTmin values also grouped into high altitude (Clarence Lagoon and Mt Wellington) and low altitude (Orford and Hobart) sites. All other combinations were significantly different ($P < 0.05$). Therefore high and low altitude populations give birth to young that are pre-adapted to their thermal environment, even when these young are gestated under identical thermal conditions.

5.3.4 Thermal sensitivity of sprint speed and willingness to run

Sprint speed was initially analysed using a factorial repeated measure ANCOVA design, with SVL and weight individually as the co-variates and study animal as the repeated factor. The importance of the co-variate was assessed; however, in each case it was not significant ($P > 0.2$). I therefore continued the analysis using factorial repeated measures

ANOVAs for both the sprint speed and willingness data. Sprint speed data for male and female animals from all study sites are displayed in Figures 5.8 and 5.9.

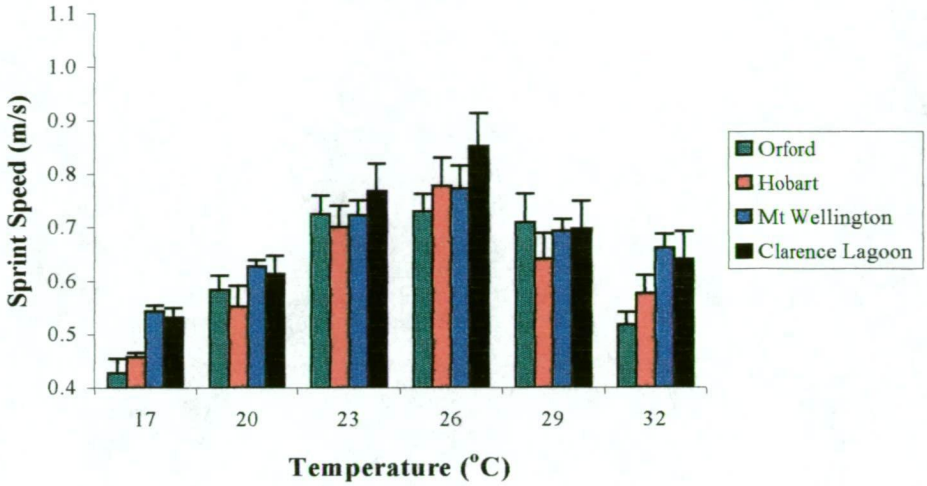

Figure5.8 Mean sprint speed of male *N. metallicus* from the high altitude (Clarence Lagoon and Mt. Wellington) and low altitude (Hobart and Orford) study sites at specified temperatures. All values represent means (± s.e.).

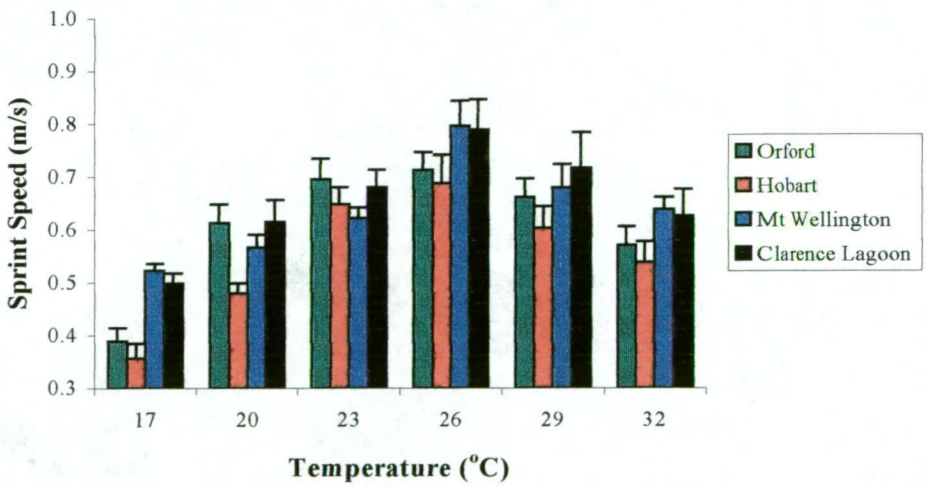

Figure5.9 Mean sprint speed of early vitellogenic non-pregnant female *N. metallicus* from the high altitude (Clarence Lagoon and Mt. Wellington) and low altitude (Hobart and Orford) study sites at specified temperatures. All values represent means (± s.e.).

Both male and female animals displayed significant site versus temperature interaction effects (male $F_{15,200} = 3.07$, $P = 0.0003$, female $F_{15,165} = 5.20$, $P = 0.0001$). The main *post-hoc* examinations of interest are the comparisons between sites at any given temperature.

These comparisons were performed using a GLM procedure. Significant variation between sites ($P < 0.05$) was evident only at 17°C and 32°C in males. In both cases, male animals from high altitude sites ran faster at extreme temperatures. Females displayed a similar trend; however, high altitude animals at 17°C were faster ($P < 0.05$) than low altitude females and animals from Hobart ran slower than animals from other sites at 20°C. Females at higher temperatures displayed no significant difference in running speed ($P > 0.05$). This may, however, reflect the large variance associated with these temperature.

Willingness to run (measured as stopping frequency) in male and female animals at the temperatures used for sprinting is shown in Figures 5.10 and 5.11. In both cases a significant site versus temperature interaction was again evident (male $F_{15,200} = 3.22$, $P = 0.0005$, female $F_{15,165} = 4.17$, $P = 0.0001$).

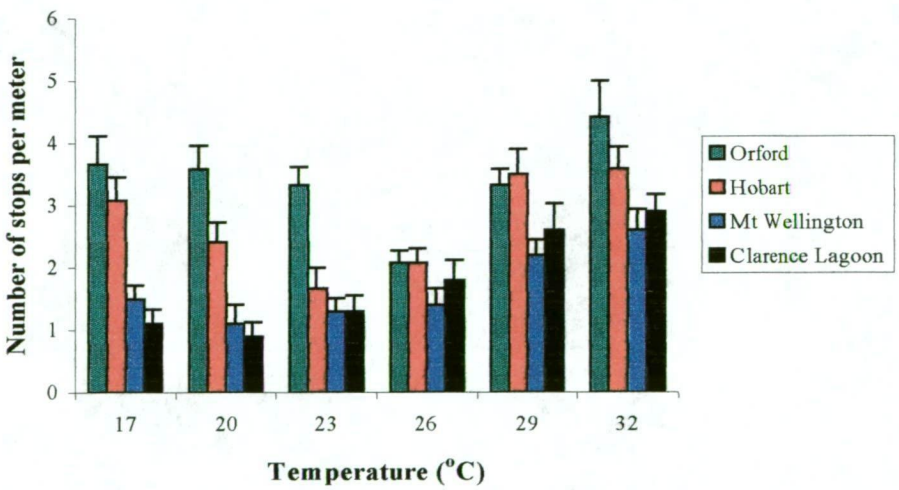

Figure5.10 Willingness to run in male *N. metallicus* from the high altitude (Clarence Lagoon and Mt. Wellington) and low altitude (Hobart and Orford) study sites at specified temperatures. All values represent means (± s.e.).

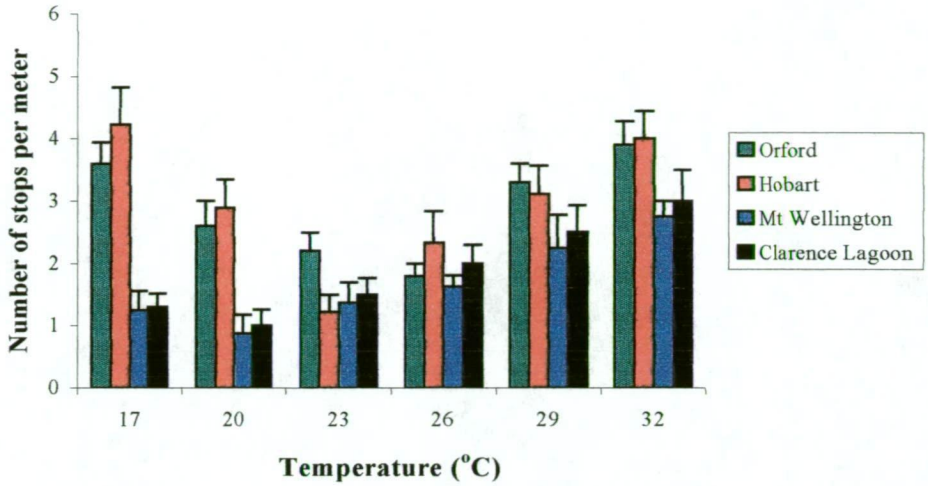

Figure5.11 Willingness to run in early vitellogenic non-pregnant female *N. metallicus* from the high altitude (Clarence Lagoon and Mt. Wellington) and low altitude (Hobart and Orford) study sites at specified temperatures. All values represent means (± s.e.).

The main *post-hoc* examinations of interest are again the comparisons between sites at any given temperature. These comparisons were also performed using a GLM procedure. The results of these analyses for both male and female *N. metallicus* are presented in Table 5.9.

Table 5.9 Results of *post-hoc* analysis (GLM procedure) examining variation in the willingness to run in animals from the four study sites at each of the indicated temperatures. Significant variation is indicated by bold type in the probability column. Significance is accepted at the 0.05 level.

Temperature	Sex	*F*(df)	Probability
17°C	**Male**	12.08(3,40)	**0.0001**
	Female	15.73(3,33)	**0.0001**
20°C	**Male**	15.19(3,40)	**0.0001**
	Female	8.26(3,33)	**0.0003**
23°C	**Male**	12.15(3,40)	**0.0001**
	Female	2.32(3,33)	0.093
26°C	**Male**	1.6(3,40)	0.204
	Female	0.84(3,33)	0.480
29°C	**Male**	3.16(3,40)	**0.035**
	Female	1.34(3,33)	0.279
32°C	**Male**	3.65(3,40)	**0.020**
	Female	2.24(3,33)	0.102

It is apparent from Figures 5.10 and 5.11 that high altitude animals generally stop less frequently than low altitude animals while sprinting. This is most obvious at low temperature and is found in both males and females. At higher temperatures, high altitude males, especially Mt Wellington specimens, also appear to stop less frequently than low altitude animals; however, no significant difference is evident in females. Also of interest from Figures 5.10 and 5.11 are the differences in temperatures at which animals stop least while sprinting. Regardless of sex, animals from Orford stop least at 26°C, animals from Hobart stop least at 23°C and high altitude animals (Clarence Lagoon and Mt Wellington) stop least at 20°C. Thus temperature and site of collection both appear to influence an animal's willingness to maintain a sprint burst.

5.4 Discussion

Individual *N. metallicus* from all sites maintained a T_b between 29°C and 31°C. It appears, therefore, that T_b is evolutionarily conservative in this species. This is not unexpected, despite the obvious differences in thermal characteristics between field sites (Chapter 2). Animals from all sites displayed considerable precision in their regulation of T_b. This observation, combined with the fact that animals from high altitude sites are able to reach high T_bs even under quite adverse conditions, indicates that no strong selection pressures are acting on this aspect of thermal biology. Christian (1998) showed that the short-horned lizard *Phrynosoma douglassi* was able to maintain T_bs around 30°C even in air temperatures of 1.5°C. In contrast, several other studies have found that intraspecific field T_bs do vary with altitude (Crowley, 1985; Van Damme *et al.*, 1989, 1990; Grant and Dunham, 1990; Smith *et al.*, 1993; Smith and Ballinger, 1994d; Spencer and Grimmond, 1994). However, other species or groups show a pattern similar to *N. metallicus* (Burns, 1970; Aldoph, 1990; Smith and Ballinger, 1995; Brown, 1996). It appears than *N. metallicus* is able to exploit microhabitats and use flexible basking strategies (Chapter 4) to minimise variation in T_b between thermally distinct habitats.

The most pronounced change in thermal environment associated with altitude at temperate latitudes is a decrease in air temperature. This is also true of sites used in this study (Chapter 2). However, mean T_a estimates for basking lizards in this study do not follow this trend. This reflects the selection of days with maximum temperatures between 19°C and 25°C. While T_a values for Mt. Wellington are lower than those for the low altitude

sites, those for Clarence Lagoon are not. This probably reflects the fact that Clarence Lagoon is sheltered from wind exposure, while on Mt. Wellington wind speeds are high as the site sits near the exposed summit. These winds would act to decrease the T_a readings associated with any given T_b value. Higher mean T_as at Orford also reflect the fact that this site has relatively little tree cover, allowing air temperatures to rise above those at shaded sites (Hobart). The pattern observed between sites for T_s is expected. Values are generally higher at low altitude. Again relative lack of tree cover may result in higher T_s values at Orford than at other sites.

The relationships evident between T_b and T_a, and T_b and T_s are useful for examining thermoregulation at each site; however, see Hertz *et al*, (1993). The T_b and T_s relationship produced equal slopes; however, examination of least square means indicated that high altitude animals maintain their T_b at lower T_s values. This, together with the low slope of the relationship (common slope = 0.298), indicates that at all sites *N. metallicus* thermoregulates effectively to maintain a stable T_b, and that this is especially true at high altitude. A more complex relationship is evident between T_b and T_a. Here the slopes of regression lines are shallower for complex forested sites, indicating that animals exposed to these habitats are better able to control T_b, than animals from habitats with little tree cover. Examinations of y-intercept values also support this conclusion. Similarly the results of the multiple regression study showed that habitat complexity significantly influenced the relative importance of independent variables (T_a and T_s) on the dependant variable T_b. In animals from complex sites (Hobart and Clarence Lagoon) T_b was influenced more strongly by T_s, while in animals from open sites T_a was generally more important.

Superimposed on this trend is a gender effect. Females appear less able to maintain T_b over the range of T_as encountered in the field (high value of slope of regressions). However, R-squared estimates from each site, which are higher in females, indicate that this sex displays a high degree of precision in control of T_b over a range of T_as. Similarly the results of the multiple regression showed that females had higher R-squared values and thus controlled T_b more tightly around the resulting models. These results indicate that thermal control is a complex phenomenon in *N. metallicus* populations, involving aspects of altitude, habit complexity and sex.

A more effective means of studying thermal biology in the field in *N. metallicus* would be to examine operative temperatures (through measuring the temperature attained by hollow-bodied copper replicas of lizards) at basking sites and at random locations in each of the four study sites (Hertz *et al.*, 1993). Hertz *et al*, (1993) identified a number of weaknesses in studying thermal biology using the methods described in this chapter, specifically in using regression slopes and variability of to T_b to evaluate thermoregulation. Nevertheless, the results described above represent an introductory investigation of thermal biology and geographic adaptation in this species. A more detailed examination of temperature regulation , using the methods described above, was not possible within the time constraints of this project; however, this may be attempted in the future.

Thermal behaviour as a mechanism of adaptation in *N. metallicus* was further investigated in the laboratory. Unlike field temperature studies, which included only pregnant females, the laboratory investigation examined thermal behaviour in males and females during both spring and autumn. Thus females were compared when pregnant (spring) and not pregnant (autumn). The results indicate that animals maintain a similar T_{ave} irrespective of site, sex or reproductive status. This value was around 25°C to 26°C, and this represents a 4°C drop from the temperatures recorded for animals in the field. While this seems difficult to explain, it must be remembered that T_{ave} recorded in the laboratory represents a measure of mean temperature of all activities, while field values are only indicative of basking periods. Thus, it is possible that over the course of the day, *N. metallicus* does allow its body temperature to average out at values less than those recorded in the field. Kik (1998), in a study of a sister species, *N. microlepidotus*, found that T_{ave} estimates in the laboratory varied between 22.4°C and 24°C, while field T_bs were significantly higher, most notably in males, in a study by Kabat (1999). While T_{ave} showed no variation between groups, other aspects of thermal behaviour did differ. These differences were recorded between sites only for minimum body temperatures and lower setpoints in males (interacting factors). In both cases high altitude animals appeared to limit their time at lower temperatures in late spring. The differences, however, were very small. Conversely, season of collection was more important in regards to time spent at extreme temperatures. Males spent less time with body temperatures above 35°C and below 20°C in spring than in autumn, possibly indicating a willingness to search for females in autumn when mating is initiated. Females displayed a more complex pattern in relation to collection dates. They spent less time at

extreme temperatures, and maintained a higher minimum temperature in spring than in autumn. They also allowed their maximum temperature to increase significantly in autumn and adjusted both upper and lower set point temperatures in spring to avoid thermal extremes. Thus, while there was no obvious change in T_{ave} in females between reproductive phases, other thermal variables changed significantly.

Differences displayed by females, in field thermal ecology and laboratory thermal behaviour, require further discussion. Numerous studies have shown that males and females may maintain different T_bs in the field at different sites (Pentecost, 1974; Patterson and Davies, 1978; Stevens, 1982; Sievert and Hutchison, 1989; Gillis, 1991). In viviparous species these differences are usually interpreted in terms of reproductive requirements of developing young (Gillis, 1991; Andrews *et al.*, 1997). Temperature significantly affects the rate of development of reptilian embryos (Muth, 1980; Ellner and Beuchat, 1984). However, temperatures above a critical value can damage offspring (Mathies and Andrews, 1997). The body temperature of female reptiles during pregnancy has been studied in a number of species of lizard and snake. However, changes in T_b associated with gestation are not consistent (Stewart, 1984; Hailay *et al.*, 1987; Van Damme *et al.*, 1987; Heulin, 1987; Schwarzkopf and Shine, 1991; Daut and Andrews, 1993). There appears to be no obvious phylogenetic or ecological correlation with a change in body temperature during pregnancy. Kik (1998) found that *N. microlepidotus* females decreased their T_{ave} by about 1.6°C when pregnant. This species also showed a decrease in time spent at extremely high temperatures. However, it appeared to spend longer periods at cooler temperatures during gestation and lowered both its thermal setpoint temperatures. Thus, *N. microlepidotus* displays dramatically different thermal behaviour during gestation than does *N. metallicus*, which shows no drop in T_{ave} and avoids both high and low temperatures by uncoupling upper and lower set point temperatures. This may be explained in terms of the reproductive biology of both species. *Niveoscincus microlepidotus* is a biennial breeder and retains developed young over winter. At the same site (Mt Wellington) the annual reproducing *N. metallicus* must give birth to young well in advance of winter. Maintaining a relatively high temperature in this species presumably maximises the rate of embryonic development and minimises any compromise between the conflicting pressures of maternal and embryonic metabolism (Tosini and Avery, 1996, Beuchat and Ellner, 1987).

Field and laboratory data on the precision of thermal control in female *N. metallicus* appear slightly contradictory. Field regression slopes indicate that pregnant female *N. metallicus* are less able to maintain high T_bs during cooler periods than are males, although they appear to regulate T_b more precisely (higher R-squared values of both linear and multiple regressions). However, laboratory data indicate that they maintain thermal control more effectively than non-pregnant or male animals (based on avoidance of thermal extremes). If animals in the field are seeking to maintain their body temperatures near maximal levels, a plausible hypothesis considering that females appear to bask more often and for longer periods when pregnant (personal observation), then any regression slope against air temperature will be high as maximal operative temperatures are linearly dependent on air temperature (Van Damme *et al.*, 1987). Thus, regression slopes alone cannot discriminate between this strategy and thermoconformity, which also results in higher regression slope values. Laboratory data therefore appear to be a more instructive method of examining thermal control, at least in *N. metallicus*, however operative temperature (T_o) evaluation in the field would represent a more powerful method of examining thermal control in female animals (Hertz *et al.*, 1993).

Data clearly indicate that *N. metallicus* actively regulates its T_b over a wide range of altitudes and that this species also accepts a wide range of temperatures during activity. In many species, behavioural shifts are the primary mechanism that compensate for geographic changes in thermal environment (Hertz and Huey, 1981; Hertz and Nevo, 1981; Huey, 1982; Van Damme *et al.*, 1989). Behavioural mechanisms have indeed been identified in *N. metallicus* as a primary means of maintaining constant preferred body temperatures between sites (Chapter 4). While behavioural mechanisms help resist microevolutionary adaptation of body temperature over altitudinal ranges, other thermal characteristics may be less resistant to selection. For example, studies examining thermal preferenda and tolerances of geographically widespread populations of the same species often find the two factors to be evolutionarily uncoupled. Animals from colder sites often have lower CTmin values (Spellerberg; 1972; Wilson and Echternavcht, 1987). CTmax has also been shown to increase in some populations exposed to high temperatures (Miller and Packard, 1977; Hertz, 1979; Hertz and Huey, 1981; Huey and Bennett, 1987; Schwarzkopf, 1998). However, animals exposed to sites with differing thermal regimes may compensate behaviourally to maintain a T_b, even while natural selection acts to increase CTmax or decrease CTmin (Hertz and Huey, 1981; Schwarzkopf, 1998).

The two remaining methods of physiological adaptation available to reptiles, acclimatization and genetic adaptation, were investigated in *N. metallicus* through examination of direct and indirect thermal performance measures (critical thermal limits and running behaviour). CTmax values were not different between sites within any collection period. Thus only season appeared to influence CTmax values and only to a very small extent. CTmin, however, was more informative. Animals from high altitude sites had significantly lower CTmin values than low altitude animals. There was also a seasonal effect apparent in males from low altitude. No evidence for acclimatory adaptation to altitude, as described by Garland and Aldoph (1991) was evident in *N. metallicus*. Differences in CTmin and CTmax values were either maintained over the four months that animals were held in the laboratory, or matched changes in animals collected and tested in autumn.

The fact that CTmin displays adaptation to altitude, while CTmax does not, indicates that these thermal characteristics are independent of each other at the genetic/physiological level. A tendency for populations from cooler environments to have lower CTmin values has already been described. Wilson and Echternacht (1987) reported that *Anolis carolinensis* displayed CTmin differences of 3.2 ± 0.45 °C in populations from different latitudes and altitudes. Brown (1996) also reported a difference of 3.9 ± 0.47 between populations of the gecko *Tarentola boettgeri*. In addition Spellerberg (1972) reported that members of a *Sphenomorphus* species group had different tolerances to low temperature that correlated with elevational zonation. *Niveoscincus metallicus* shows similar variation in CTmin to these species, indicating that this characteristic is evolutionarily plastic and represents an important aspect of the thermal biology of the species.

Sprint speed and stopping frequency (performance measures) were also recorded for male and female animals during autumn only. Thus no pregnancy correlations were studied in relation to either of these variables. Irrespective of site both males and females sprint fastest at around 26°C. Significant differences were recorded for animals at low temperatures (17°C) and high temperatures (32°C) in males. Generally high altitude animals ran faster at extreme temperatures. Willingness to run produced even clearer results. The greatest willingness to sprint occurred at 20°C for high altitude animals, at

23°C for animals from Hobart and at 26°C for Orford animals, irrespective of sex. Animals from high altitude sites also refused to run significantly less frequently than low altitude animals at colder temperatures, while males also stopped less often at higher temperatures. Previous studies have reported that performance breadth correlates with the variability in field T_bs among species of anoles (van Berkum, 1986) but not among populations within a species (*Stellio stellio*: Hertz *et al.*, 1983; *Sceloporus undulatus*: Crowley, 1985; *Sceloporus occidentalis*: van Berkum, 1988; *Podarcis tiliguerta*: Van Damme *et al.*, 1989). In contrast, *N. metallicus* displays a rather different response. T_bs recorded in the field are effectively controlled by behavioural mechanisms between 29°C and 31°C, irrespective of altitude (see above). However, animals anywhere in Tasmania can expect periodical exposure to very cold conditions. High altitude populations appear to have adapted to this problem by expanding the thermal breadth of their sprint performance, thus allowing them to maintain relatively high speeds at lower temperatures. This, combined with a greater willingness to keep running under cool conditions at high altitude, represents a significant adaptation to high altitude environments.

As *N.* metallicus accepts such a wide range of temperatures when active and is able to perform well over much of this range, it may be possible that selection pressures for adjustment of thermal performance are weak. Nevertheless, the differences observed between populations in regard to tolerance of low temperatures and sprint performance are unlikely to represent acclimatization to thermal conditions. This is because acclimation of thermal limits was not evident in animals held in the laboratory for 4 months. Furthermore, juvenile animals born in the laboratory to mothers housed together during gestation still displayed differences in thermal limits consistent with their origins. Several species have been found to shift their CT limits after exposure to low temperatures (Raglund *et al.*, 1981; Tsuji 1986 in van Berkum, 1988). However, the magnitude of these shifts (usually 1°C – 2°C) is smaller than that observed in *N. metallicus* (this study) and in other species where acclimation has been discounted (van Berkum, 1988). Thermal history has been shown to affect susceptibility of ectotherms to high temperatures in laboratory acclimation studies. For example *Eulamprus quoyii* acclimated to 30°C had higher lethal temperatures than those acclimated to 20°C (Veron and Heatwole, 1970). However, CTmin did not differ between high and low altitude laboratory acclimated populations of the African skink *Mabuya striata* (Patterson, 1991). In addition the limited data available imply that sprint speed is insensitive to previous acclimation history (van Berkum, 1988).

Although Bennett (1980) reported significantly lower absolute sprint speeds in winter collected *Sceloporus occidentalis* than in summer collected animals, these collections did not differ in relative speed. *Anolis humilis* also showed no change in sprint speed when held under differing thermal regimes (van Berkum, 1986). Thus it seems improbable that differences in sprint performance are the result of acclimatization in the field to high altitude.

A second type of adaptive response also merits consideration. The possibility exists that developmental conditions, during gestation and early post-natal growth, may fix thermal tolerance values and performance characteristics. Thermal environment during gestation is known to affect sex at birth (Harlow and Shine, 1999), as well as many aspects of juvenile phenotype (Downes and Shine, 1999; Shine and Downes, 1999; Brãna and Ji, 2000; Swain and Jones, 2000b). Qualls and Shine (1998) also indicated that environment during embryo development and during early life can affect aspects of an animal's phenotype. Modification of physiological systems in response to different thermal environments during development to adult size has not been examined in reptiles. However, juvenile CT limits reported here, for mothers held under common conditions throughout gestation, indicate that young are born already in possession of many of their thermal characteristics (i.e. thermal tolerances) and therefore these characters may have a significant genetic basis. While mothers were held under constant conditions, the possibility exists that gestation environment, influenced by variation in thermal behaviour, did vary between population groups. However, laboratory thermal characteristics (section 5.3.2) indicate that pregnant females display no site induced variation in thermal behaviour in the laboratory. Thus, differences in CT limits in laboratory gestated juveniles are unlikely to reflect variation in gestation environment.

Two conflicting opinions have been identified by Hertz *et al.* (1983) concerning the genetic basis of reptilian thermal adaptation. The 'labile' view states that when environmental conditions induce a shift in active body temperatures, parallel changes in thermal optima should evolve readily. Partial co-adaptation between active T_bs and thermal optima has indeed been documented in inter-specific studies of anoline (van Berkum, 1986) and scincid lizards (Huey and Bennett, 1987). The alternative 'static' view of thermal adaptation claims that thermal physiology is evolutionary inert and resistant to directional selection. Support for this view is provided by studies of the thermal sensitivity

of sprint speed among conspecific lizard populations inhabiting thermally distinct habitats (Hertz *et al.*, 1983; Crowley, 1985; Van Damme *et al.*, 1989). In contrast to these studies, my research supports the 'labile' position for both thermal sensitivity of sprinting and willingness to sprint in *N. metallicus*, and more obviously for thermal tolerance.

This conclusion requires some explanation. Active T_bs in *N. metallicus* appear to be under stabilizing selection; however, animals occupying extreme environments will inevitably be exposed to near lethal temperatures on occasion. The gradual exposure to colder conditions, assuming evolution of this species from a low altitude form (Melville and Swain, 2000b), would have required increasingly greater tolerance of low temperatures, and thus permanent adaptation of CTmin. This is common in many species. However, adaptation of thermal performance is apparently uncommon. *Niveoscincus metallicus* appears to display a direct connection between performance and overall fitness. Thus, locomotory handicaps must have been sufficient for animals to have evolved the ability to maintain relatively fast sprint speeds at lower temperatures. This capacity would be especially important in spring and autumn, when high altitude environments offer poor thermal opportunities (Chapter 2). Consequently, adaptation of thermal performance may have evolved in this species because the short season open to high altitude animals may reduce opportunities for vital activities such as foraging. However, if animals are able to remain active at reduced temperatures then ecological performance would be little decreased. Assuming that ecological performance (feeding and predator avoidance) and physiological performance (sprint speed) scale directly (Huey and Stevenson, 1979; Christian and Tracy, 1981), pressure to increase speed at low temperatures would be great. Also many studies have identified compensatory increases or decreases in behaviour (such as alertness) that may reduce the costs of decreased speed to predator escape (Brodie and Russell, 1999). However, *N. metallicus* at high altitude appear less wary than low altitude conspecifics (personal observation), and thus the fact that they allow predators to approach closer at high altitude may again increase the need to run faster at low temperatures. Therefore, this species may have adapted thermal performance, while other species living at high altitude, subject to lower predation or food collection pressures, may have had no need to evolve increase speed or willingness to sprint at lower T_bs (Crowley, 1985; Van Damme *et al.*, 1989).

Hertz *et al.* (1983) suggested that an ability to run quickly over a broad range of temperatures may contribute more to an ectotherm's fitness than an ability to be very quick over a narrow range of temperatures. *Niveoscincus metallicus* appears to have adopted this strategy at high altitude, but not at low altitude. Despite the fact that T_b, and many other thermoregulatory characteristics, appear to be under stabilising influences in this species, thermal adaptation has occurred across the range of altitudes occupied. Thus, critical thermal limits and activity at extreme temperatures appear to have evolved independently of preferred temperature in *N. metallicus*.

CHAPTER SIX

Geographic and annual life history variation in *Niveoscincus metallicus*

6.1 Introduction

Stearns (1976) defined life history tactics as a "series of co-adapted traits designed, by natural selection, to solve particular ecological problems". Species with broad geographic ranges often display extensive variation in life history traits. This variation occurs primarily at two levels: variation on a geographic scale, between populations of a single species (Dunham*et al.*, 1988; Grant and Porter, 1992; Taylor *et al.*, 1992; Ferguson and Talent, 1993; Niewiarowski and Roosenburg, 1993; Niewiarowski, 1994; Forsman and Shine, 1995; Sorci and Clobert, 1999; Wapstra and Swain (in press); and variation on an annual or seasonal basis within a single population (Dunham, 1978; Ballinger and Congdon, 1980; Schwarzkopf, 1992; Smith *et al.*, 1995; Olsson and Shine, 1997; Abell, 1999; Wapstra and Swain (in press). An understanding of the life history of an organism, and specifically of variation at each of the above levels, is of paramount importance to any investigation of adaptation to environmental extremes.

All animals must make a number of "decisions" about aspects of life history. For any given reproductive event a female faces two major allocation "decisions". Firstly, she must determine how much energy to invest in the reproductive event (reproductive effort), and secondly she must decide how much energy to invest in each offspring (parental investment) (Winkler and Wallin, 1987). Life history models suggest that at any point in an individual's life there is an optimum proportion of available energy that should be diverted to reproduction (Williams, 1966; Forsman and Shine, 1995). This amount of energy is thought to reflect trade-offs, particularly between current and future reproduction (Shine and Schwarzkopf, 1992; Forsman and Shine, 1995), and between clutch size and parental investment (offspring size) (Lessells, 1991; Madsen and Shine, 1992; Roff, 1992; Stearns, 1992; Olsson and Shine, 1997; Abell, 1999). Life-history traits such as reproductive effort should therefore evolve so as to maximise the total lifetime fecundity of an individual (Shine and Schwarzkopf, 1992).

Variation in allocation "decisions", however, result from two main sources: (i) genetic, including phylogenetic influences, adaptation to local conditions and environments, and individual variation in genotype; and (ii) proximate, including variation generated by factors external to the individual itself (Smith, 1998). Thus environment can act to constrain reproductive effort through both phenotypic expression of the genotype during ontogeny, and, ultimately as a source of selection on the life history genotype itself (Niewiarowski and Roosenburg, 1993). It is widely appreciated that to fully understand evolution of life history and geographic variation in life history, one must attempt to disentangle genetic and environmental variation in natural populations (Stearns, 1980; Ballinger, 1983; Jones and Ballinger, 1987; Schwarzkopf, 1992; Adolph and Porter, 1993; Niewiarowski and Roosenburg, 1993; Sorci *et al.*, 1996; Qualls and Shine, 1998).

Phenotypic plasticity is the differential phenotypic expression of a given genotype across differing environments or time periods, and represents one of the most acknowledged sources of variation in life history phenotypes (Roff, 1992; Stearns, 1992; Sorci *et al.*, 1996). Among reptiles, temperature, food availability and habitat occupation have all been shown to affect phenotypic expression of a genotype (Ballinger, 1977; Dunham, 1978; Jones *et al.*, 1987; Sinervo and Adolph, 1989, 1994; Niewiarowski and Roosenburg, 1993; Sorci *et al.*, 1996; Olsson and Shine, 1997; Smith, 1998; Qualls and Shine, 1998; Abell, 1999; Swain and Jones; 2000b; Wapstra, 2000; Wapstra and Swain; in press). Adolph and Porter (1993, 1996) have produced models examining the effect of temperature on various aspects of life history. Their results demonstrate that patterns of life history variation can be described without invoking genetic differentiation. This clearly shows the potential of proximate factors to influence reptilian life-history phenotypes.

The high degree of phylogenetic conservatism in reptilian life histories (Stearns, 1984; Dunham *et al.*, 1988; Bauwens and Diaz-Uriate, 1997) means that, while a large body of literature does exist pertaining to life history evolution (Ballinger, 1973; Ferguson *et al*, 1980; Jones and Ballinger, 1987; Jones *et al.*, 1987; Sinervo and Adolph, 1989; Sinervo 1990; Ferguson and Talent, 1993; Niewiarowski band RoosenBurg, 1993; Sorci *et al.*, 1996; Bauwens and Diaz-Uriarte, 1997; Smith, 1998; Abell, 1999), independent data sets are still required for species from other phylogenetically distinct taxa, in order to fully appreciate relationships between life history characteristics and environmental variables

(Dunham *et al*,. 1988; Forsman and Shine, 1995; Wapstra and Swain, in press). Since geographic variation in life-history phenotypes between populations of a single species may reflect both genetic divergence and phenotypic plasticity induced by environmental variation, simple examinations of variation between sites alone provides little information on causation of observed differences. In addition, variation can also occur over time within a single population (Schwarzkopf, 1992; Olsson and Sine, 1997; Pamula, 1997; Wapstra and Swain, in press). This annual variation in life-history characteristics is thought to reflect primarily proximate variation from environmental fluctuations (Schwarzkopf, 1992). Thus examination of annual variation helps shed light on some of the constraints influencing life history tactics at the microevolutionary level (Schwarzkopf, 1992; Olsson and Shine, 1997).

The aim of this section of work was to describe variation in some aspects of life history (adult body size, reproductive effort, clutch size and parental investment) in *N. metallicus* between my four field sites. These sites cover the altitudinal range of the species and may also provide information on the influence of habitat on life history. Because animals living in differing habitats are exposed to differing proximate constraints (i.e. abundance and availability of food (Brown *et al.*, 1994), foraging efficiency (Diehl, 1993), temperature (Huey, 1991; Chapter 2) and predation risk (Christian and Tracy, 1981; Chapter 4), life history variation between sites may be affected by many factors other than altitude. The study also examines variation over a three-year period (1997-1999) so as to provide information on annual variation in selected life-history characteristics within populations. Six specific life history questions were identified.

1) Does adult body size vary between populations, and how does any such variation correlate with environmental variables?

2) Do litter sizes, mass or relative clutch mass vary between populations?

3) Do offspring size and condition vary between populations?

4) Is there evidence of variation in sex ratios of offspring between populations?

5) If so, is there any evidence for differential allocation of resources to sexes?

6) Does inter-annual variation exist in any of the above life history characteristics within populations?

6.2 Materials and Methods

6.2.1 Field collections

Animals were captured as part of a general study of life history adaptation from the four field sites identified in Chapter 2. They were brought into the laboratory where the following parameters were measured: snout-vent length (SVL)(± 0.1 mm); mass (± 0.1 mg); total length (± 0.1 mm); and if present, position of tail break (± 0.1 mm). Amimals were sexed by eversion of hemipenes (if present). If animals were not required for any other aspect of work they were subsequently released at the site of capture. Other animals were killed by placing them in a freezer at –20°C (Cogger, 1992).

6.2.2 Data collection

Litter size was determined using two methods: autopsy and live birth. Despite the low levels of atresia of vitellogenic follicles in *N. metallicus* and the general lack of variation between counts of corpora lutea and embryos in pregnant animals (Jones and Swain, 1996), data from animals with early follicular development were not included in any autopsy data. Only animals near ovulation or carrying embryos were included in the data set.

Litter size was also calculated from live births in the laboratory. Collections of near-term gravid females were made late in the reproductive season at all four sites during 1998 and 1999. During 1997 animals were collected from all sites except Orford. Females were held under standard laboratory conditions (Chapter 2) in groups of two animals until parturition occurred. Cages were checked twice daily for newborns. Farr and Gregory (1991) suggested that holding reptiles (snakes) in captivity can influence juvenile phenotype and recommended holding mothers for as short a period as possible before birth in the laboratory. Therefore litter and neonate data were recorded only for animals held in the

laboratory for less than 2 weeks. To meet this criterion low altitude animals were collected from late December to early January, while high altitude mothers were collected in early February. Females were removed from cages following birth and killed at –20°C. Newborns were either killed, or, if required for another aspect of research, SVL (± 0.1 mm), total length (± 0.1 mm), and mass (± 0.01 mg) were measured. Following this they were released at the site of capture of mothers. All mothers were weighed, and abdominal fat body mass was recorded. Abdominal fat body mass and sex were also recorded for newborns that were killed. Sex could only be determined reliably from dissection in *N. metallicus* offspring. For all young, body condition (mass/SVL) and relative tail length (shape = total length/SVL) were also calculated. Litter mass was defined as the total mass of all offspring in a litter, and relative clutch mass (RCM) was defined as:

RCM = litter mass / mass of postpartum female.

Relative clutch mass (RCM) can be calculated as the ratio of litter mass to either maternal gravid mass, or postpartum mass. The former method, while commonly employed (Vitt and cogdon, 1978; Vitt and Price, 1982), may introduce statistical artifacts because clutch mass is included in both numerator and denominator (Shine, 1980; Sinervo *et al.*, 1991; Forsman and Shine, 1995; Wapstra and Swain, in prep). Thus it was not used in this investigation.

6.2.3 Data analysis

All data were analysed using SAS System for Windows ©v6.12 and Systat version 7.0. Variation in adult body size (SVL) for each sex was examined using one-way ANOVA with annual variation examined within each site, and between site variation examined on total data. Male SVL was recorded only for the 1997-98 and 1998-99 seasons. Female SVL was recorded in the 1996-97, 1997-98 and 1998-99 seasons. Homoscedacity of variances was investigated by visual inspection of plots of group standard deviations versus group means. Normality of the data was evaluated through the inspection of a plot of residuals against predicted residual values. Significant interactions were further examined using Tukey's studentized range tests. Variation in SVL between sexes at each site was examined using two-sample T-tests.

Litter characteristics (Litter size, mass and RCM) were examined using Analysis of Covariance (ANCOVA), again comparing between years within each site, and secondly comparing between sites. In all cases maternal SVL acted as the covariate. The assumption that each treatment had identical group regression slopes was tested by assessing the significance of the treatment*covariate interaction. In all cases this interaction was not significant. Linearity of the relationship between the dependent variable and the covariate was evaluated by examining a plot of the residuals versus the covariate and assessing the normality of the residuals. Finally, the independence of residual variances and response variables was assessed by examining a plot of residuals against predicted values and comparing the variance of residuals among groups. *Post-hoc* LSD tests were employed to examine variation between groups.

Offspring variation between sites was initially investigated using ANCOVA, with offspring mass and SVL acting as factors and maternal mass, SVL, inter-limb length and abdominal reserves (fat bodies) acting as the covariate. Assumptions of ANCOVA were examined as described above. All offspring characteristics were also examined using one-way ANOVA, both on an inter-annual and geographic scale. Significant interactions were further examined using Tukey's studentized range tests. Assumptions of normality and homoscedacity of variances were examined as described above. No data transformation was required. Sex ratios of neonates from each site were examined using Pearson Chi-squared tests. Variations between sexes within each site for all offspring characteristics were examined using two-sample T tests.

6.3 Results

6.3.1 Adult body size

Mean adult male and female snout-vent lengths for 1996-97, 1997-98 and 1998-99 seasons for animals from all four field sites are presented in Table 6.1. Females were judged to be mature when carrying enlarged follicles or embryos during the reproductive season, or having given birth in the laboratory. Males were judged to be mature based on comparisons with sizes of mature animals determined from autopsy, or from direct autopsy.

Table 6.1 Mean adult male (M) and female (F) snout-vent lengths for animals from Orford, Hobart, Mt Wellington and Clarence Lagoon during the 1996-97, 1997-98 and 1998-99 field seasons. Values are means ± s.e. Sample sizes are shown in parentheses.

Site	1996-97	1997-98	1998-99	All years
Hobart (M)	NA	54.7 ± 0.71 (35)	54.1 ± 0.69 (30)	54.4 ± 0.49 (65)
Hobart (F)	50.8 ± 0.65 (47)	53.8 ± 0.48 (65)	52.7 ± 0.53 (55)	52.6 ± 0.33(167)
Orford (M)	NA	53.3 ± 0.56 (40)	54.8 ± 0.73 (20)	53.8 ± 0.45 (60)
Orford (F)	53.2 ± 1.22 (11)	55.1 ± 0.52 (57)	54.7 ± 0.63 (41)	54.8 ± 0.38(109)
Mt. Well (M)	NA	55.4 ± 0.44 (34)	55.6 ± 0.53 (27)	55.5 ± 0.34 (61)
Mt. Well (F)	54.4 ± 0.77 (41)	54.8 ± 0.76 (40)	55.7 ± 0.54 (57)	55.1 ± 0.39(138)
Clar Lag (M)	NA	55.5 ± 0.43 (35)	56.6 ± 0.46 (29)	56.0 ± 0.32 (64)
Clar Lag (F)	53.8 ± 0.53 (34)	55.3 ± 0.48 (53)	55.9 ± 0.33 (88)	55.3 ± 0.25(175)

Male and female size variation was initially examined within sites between years. No variation was detected between years for any of the male populations (Mt Wellington: $F_{1,59} = 0.08$, $P = 0.79$; Clarence Lagoon: $F_{1,59} = 2.76$, $P = 0.1$; Hobart: $F_{1,59} = 0.28$, $P = 0.6$; Orford: $F_{1,59} = 2.48$, $P = 0.12$). In contrast two of the female populations did show some degree of variation on an annual level (Clarence Lagoon: $F_{1,59} = 5.2$, $P = 0.006$; Hobart: $F_{1,59} = 7.25$, $P = 0.001$). During the 1996-97 season animals captured at Clarence lagoon were significantly smaller than those captured during the 1998-99 season, but not the 1997-98 season. At Hobart, animals captured during the 1996-97 season were smaller than those caught in 1997-98, but not those from 1998-99. No other variation at these sites was detected. These differences were, however, quite small and probably reflect collection

bias. Female animals captured at Mt Wellington ($F_{1,59} = 1.04$, $P = 0.36$) and Orford ($F_{1,59} = 1.12$, $P = 0.33$) showed no variation in SVL between years. Due to the fact that female variation was small and most probably reflected collection error, between site variation for both males and females was examined using data pooled from all years. The distribution of SVLs for mature females and males is shown in Figures 6.1 (a-d) and 6.2 (a-d) respectively.

Female *N. metallicus* showed differences in mean SVL between populations ($F_{3,585} = 16.23$, $P < 0.001$) (mean values, mm: Mt Wellington, 55.1; Hobart, 52.6; Clarence Lagoon, 55.3; Orford, 54.8). Animals from the Hobart site were significantly smaller than those from all other sites ($P < 0.05$). Other populations were not significantly different. Variation was also evident between male populations ($F_{3,246} = 5.81$, $P < 0.001$) (mean values mm: Mt Wellington, 55.5; Hobart, 54.4; Clarence Lagoon, 56.0; Orford, 53.8). In this case animals from Clarence Lagoon were larger than males from Hobart and Orford ($P < 0.05$), while animals from Mt Wellington were also larger than animals from Orford ($P < 0.05$). Again other combinations were not significantly different. In life history theory, the age at maturity for females is defined as the age at first birth, and not when other events (such as vitellogenesis, ovulation or mating) first occur (Stearns, 1992). In *N. metallicus* this is a significant distinction, as first mating and most vitellogenesis occur approximately 8 months before birth. Figure 6.1 presents SVLs of females which were often captured either prior to parturition or prior to ovulation. Thus size at maturity in this figure appears to be quite small, especially for low altitude sites. However, the smallest mature (at time of birth) females observed at Clarence Lagoon and Mt Wellington were 47.7 mm and 47.9 mm respectively. Corresponding values for Orford and Hobart animals were 48.1 mm and 47.2 mm respectively. Thus females may ovulate at a smaller size at low altitude, but first birth clearly occurs at a relatively constant size at all sites.

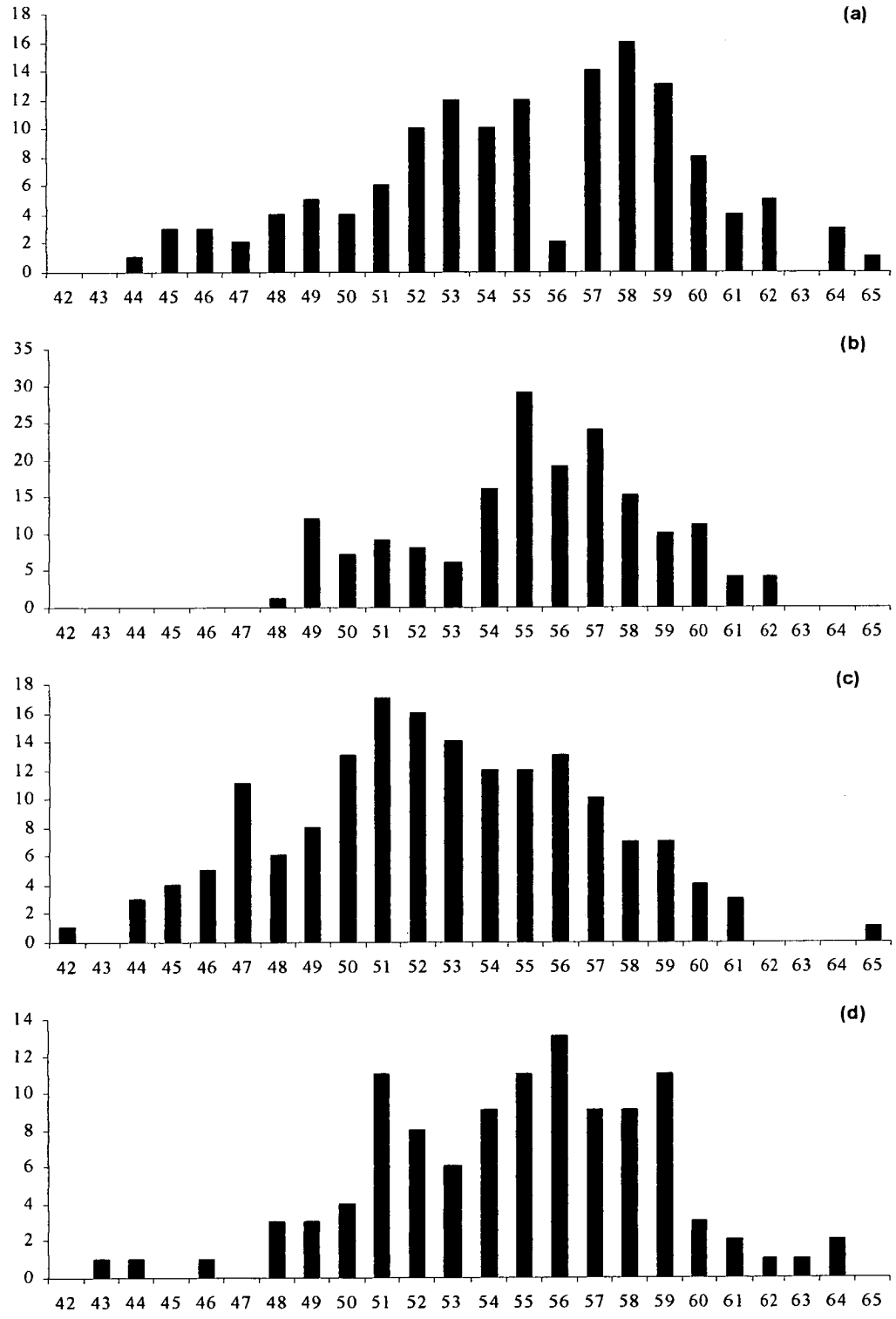

Figure 6.1. Size frequency distribution in snout-vent length for reproductive adult female *Niveoscincus metallicus* from: (a) Mt Wellington; (b) Clarence Lagoon; (c) Hobart; and (d) Orford. Data represent amalgamation of animals collected during the 1996-97, 1997-98 and 1998-99 field seasons.

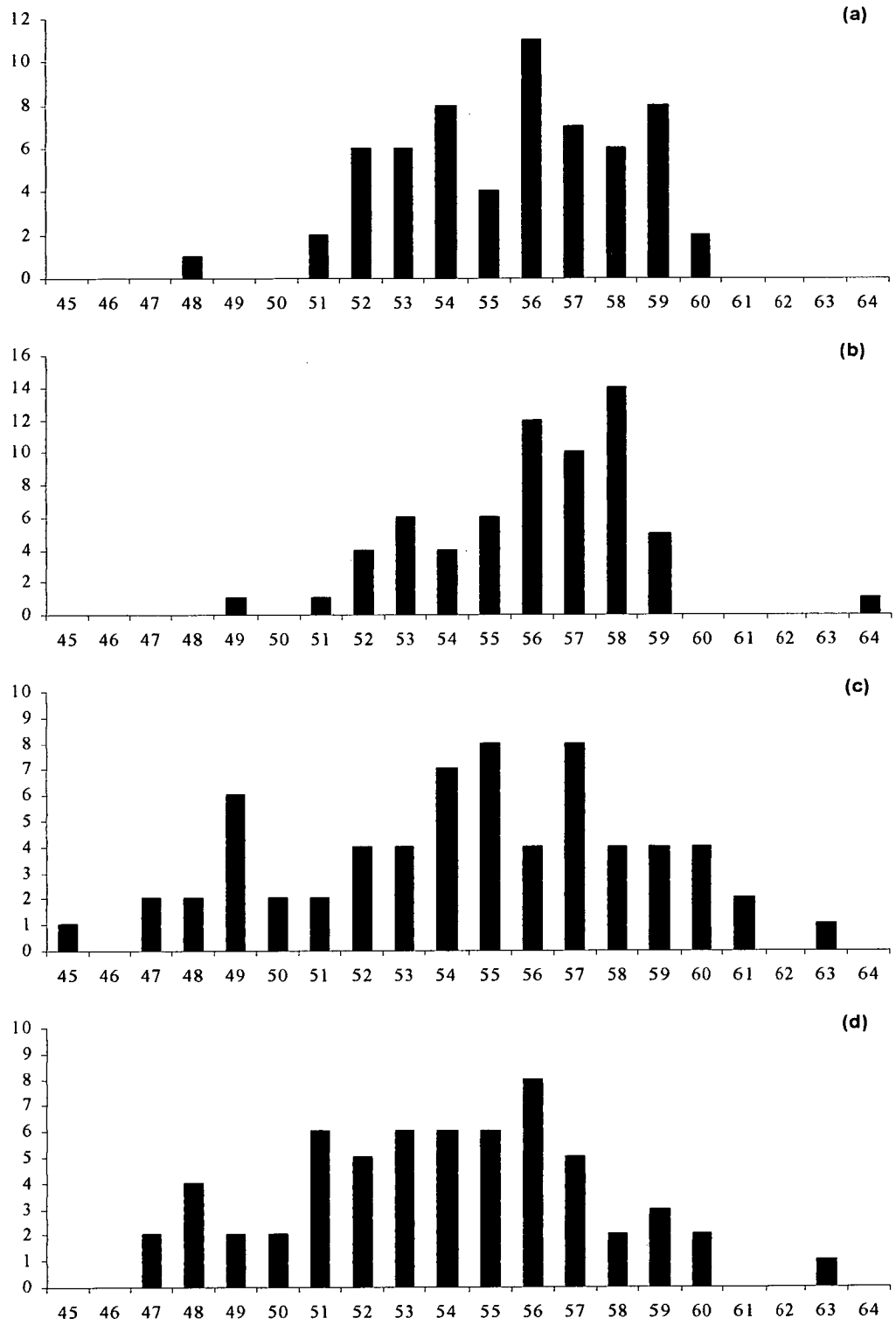

Figure 6.2. Size frequency distribution in snout-vent length for adult male *Niveoscincus metallicus* from: (a) Mt Wellington; (b) Clarence Lagoon; (c) Hobart; and (d) Orford. Data represent amalgamation of animals collected during the 1997-98 and 1998-99 field seasons.

Male and female *N. metallicus* showed significant differences in SVL at only one of the sites. Females from the Hobart site were significantly smaller than their corresponding males ($P = 0.002$) (Table 6.1). Adults could not be divided into age classes based on SVL measurements (Figures 6.1 and 6.2). Age-size relationships are examined in greater detail in Chapter 7 using skeletochronology.

6.3.2 Litter characteristics

In most cases litter characteristics were influences by maternal SVL. At all sites the relationship between maternal SVL and clutch size (slope of the linear regression) was consistent on an annual basis (Mt Wellington, $F_{2,78} = 0.12$, $P = 0.89$; Hobart, $F_{2,100} = 0.98$, $P = 0.38$; Clarence Lagoon, $F_{2,94} = 0.35$, $P = 0.70$; Orford, $F_{2,48} = 0.08$, $P = 0.93$). At all sites the slope of the relationship was significantly different from zero (Mt Wellington, $F_{1,80} = 148.1$, $P < 0.001$; Hobart, $F_{1,102} = 175.3$, $P < 0.001$; Clarence Lagoon, $F_{1,96} = 55.9$, $P < 0.001$; Orford, $F_{1,50} = 69.2$, $P < 0.001$). Regression lines (determined from ANCOVA) are presented for each year at each site in Figures 6.3 to 6.6. No interannual variation in litter size was evident at three of the four sites (Mt Wellington, $F_{2,80} = 1.11$, $P < 0.33$; Clarence Lagoon, $F_{2,96} = 3.04$, $P < 0.052$; Orford, $F_{2,50} = 1.04$, $P < 0.36$). However, differences were recorded at the Hobart site ($F_{1,102} = 3.17$, $P = 0.046$). During the 1996-97 period, litter sizes were slightly larger than in either the 1997-98 or 1998-99 seasons. Least square means of clutch size (adjusted for maternal SVL) from the above ANCOVAs are presented in Table 6.2.

Table 6.2 Least-square means of litter size for animals from Mt Wellington, Hobart, Clarence Lagoon and Orford during the 1996-97, 1997-98 and 1998-99 field seasons calculated from ANCOVAs (see text). Values are LS means ± s.e. Sample sizes are included in parentheses.

Year	Mt Wellington	Hobart	Clarence Lagoon	Orford
1996-97	3.58 ± 0.103	3.74 ± 0.114	3.36 ± 0.110	3.60 ± 0.270
	(41)	(43)	(33)	(6)
1997-98	3.43 ± 0.178	3.34 ± 0.142	3.42 ± 0.133	3.87 ± 0.133
	(14)	(28)	(22)	(26)
1998-99	3.74 ± 0.123	3.39 ± 0.125	3.08 ± 0.092	3.59 ± 0.144
	(29)	(35)	(45)	(22)

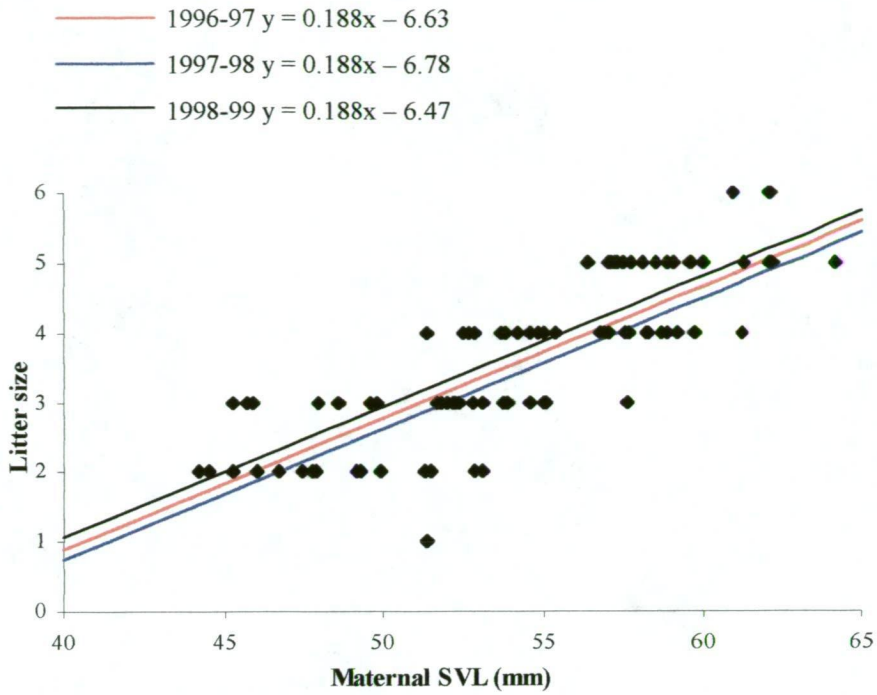

Figure 6.3 Relationship between maternal SVL (mm) and litter size in female *N. metallicus* from the Mt. Wellington field site. Regression lines are calculated from ANCOVA.

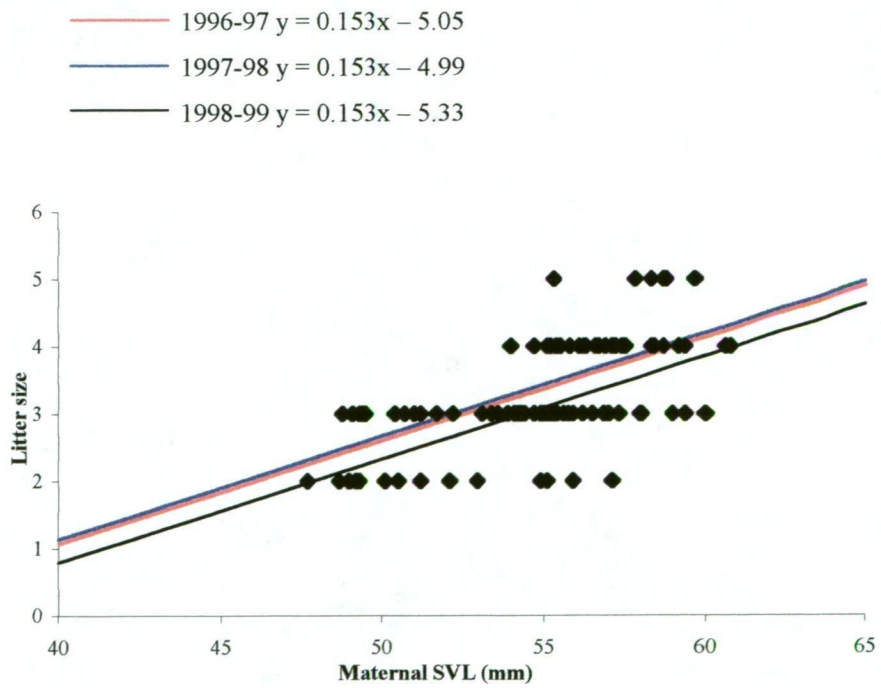

Figure 6.4 Relationship between maternal SVL (mm) and litter size in female *N. metallicus* from the Clarence Lagoon field site. Regression lines are calculated from ANCOVA.

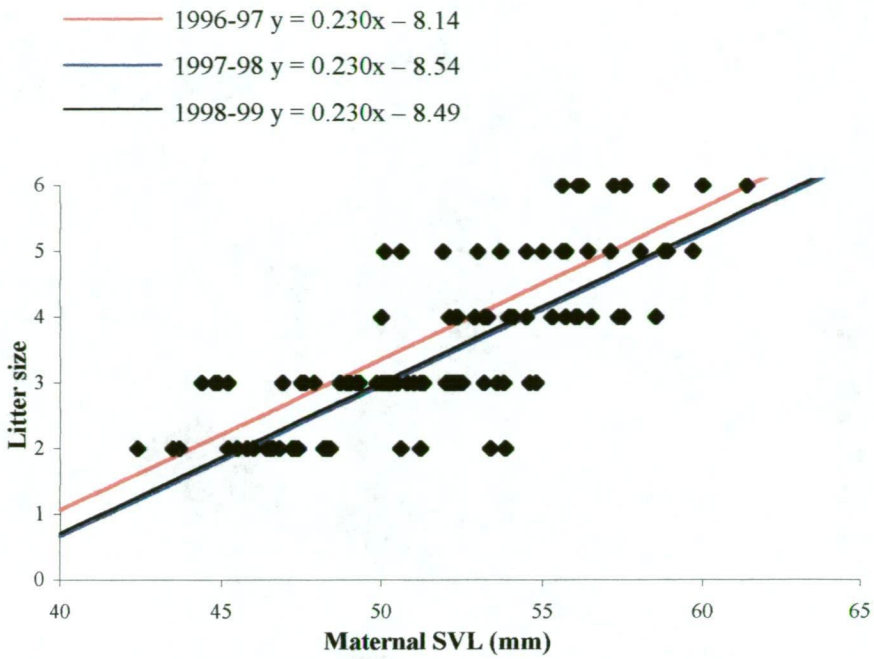

Figure 6.5 Relationship between maternal SVL (mm) and litter size in female *N. metallicus* from the Hobart field site. Regression lines are calculated from ANCOVA.

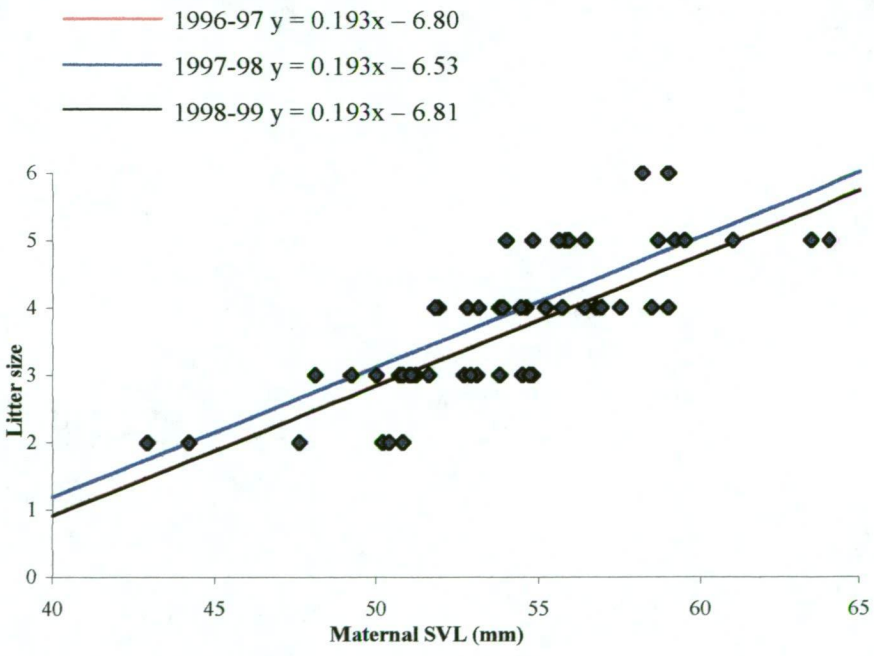

Figure 6.6 Relationship between maternal SVL (mm) and litter size in female *N. metallicus* from the Orford field site. Regression lines are calculated from ANCOVA.

The absence of annual differences in litter size for all sites except Hobart, where differences barely reached significance, allowed all within site data to be pooled for an inter-site comparison. The assumption of homogeneity of slopes was not violated ($F_{3,336}$ = 2.40, P = 0.068). Again the effect of maternal SVL on clutch size was significant ($F_{3,339}$ = 461.9, $P < 0.001$) (slope = 0.197), as was the effect of site of capture ($F_{3,339}$ = 27.11, $P <$ 0.001). Least-Square mean clutch sizes from this analysis for each site are presented in Table 6.3.

Table 6.3 Least-square means of litter size for animals from Mt Wellington, Hobart, Clarence Lagoon and Orford covering all field seasons calculated from the above ANCOVA. Values are LS means ± s.e. Sample sizes are also provided.

Site	LS mean	±SE LS mean	Number
Mt Wellington	3.5	0.08	84
Hobart	3.9	0.07	106
Clarence Lagoon	3.0	0.07	100
Orford	3.7	0.09	54

Animals from Hobart had significantly larger litters than mothers from all other sites. Litters from Mt Wellington were larger than those from Clarence Lagoon, but did not differ significantly from those of Orford mothers. Orford mothers also produced significantly larger litters than females from Clarence Lagoon. In all cases significance was accepted at the 0.05 level.

Total litter mass was also examined over a three year period within sites to evaluate environmentally induced plasticity in this characteristic. Again, at each site the requirement of homogeneity of slopes was not violated (ANCOVA: Mt Wellington, $F_{2,43}$ = 0.28, P = 0.76; Hobart, $F_{2,36}$ = 0.17, P = 0.85; Clarence Lagoon, $F_{2,42}$ = 1.09, P = 0.35; Orford, $F_{2,21}$ = 0.11, P = 0.75). Similarly, maternal SVL significantly influenced clutch mass at all sites (Mt Wellington, $F_{2,45}$ = 112.5, $P < 0.001$; Hobart, $F_{2,38}$ = 44.3, $P < 0.001$; Clarence Lagoon, $F_{2,44}$ = 20.9, $P < 0.001$; Orford, $F_{2,22}$ = 26.75, $P < 0.001$) (Common slopes = Mt Wellington =57.09; Hobart = 49.95; Clarence Lagoon = 33.31; Orford = 54.55). Season did not significantly affect litter mass at Hobart ($F_{2,38}$ = 2.08, P = 0.14), Orford ($F_{2,22}$ = 1.35, P = 0.26), or Clarence Lagoon ($F_{2,44}$ = 0.35, P = 0.71). However, seasonal variation was detected at the Mt Wellington site ($F_{2,45}$ = 8.76, $P < 0.001$). This

arose from an increased clutch mass during the 1998-99 season. Least square means of clutch mass calculations (adjusted for maternal SVL) from the above ANCOVAs are presented in Table 6.4.

Table 6.4 Least-square means of litter mass (mg) for animals from Mt Wellington, Hobart, Clarence Lagoon and Orford during the 1996-97, 1997-98 and 1998-99 field seasons calculated from the relevant ANCOVAs. Values are LS means ± s.e.. Sample sizes are provided in parentheses.

Year	Mt Wellington	Hobart	Clarence Lagoon	Orford
1996-97	672.6 ± 37.77	839.8 ± 42.42	685.6 ± 39.67	NA
	(13)	(14)	(10)	
1997-98	680.0 ± 45.27	835.8 ± 46.39	718.7 ± 32.76	968.0 ± 51.24
	(9)	(12)	(15)	(12)
1998-99	841.7 ± 25.99	730.3 ± 40.79	685.3 ± 26.34	879.2 ± 48.92
	(27)	(16)	(23)	(13)

All data were pooled within sites to examine geographic variation for this characteristic. The assumption of ANCOVA of homogeneity of slopes was not violated ($F_{3,156} = 2.00$, $P = 0.12$). Again the effect of maternal SVL on clutch mass was significant ($F_{3,159} = 215.1$, $P < 0.001$) (common slope = 52.79). Clutch mass differed between sites ($F_{3,159} = 12.48$, $P < 0.001$)(Table 6.5).

Table 6.5 Least-square means of litter mass (mg) for animals from Mt Wellington, Hobart, Clarence Lagoon and Orford derived from ANCOVA using maternal SVL as the covariate. Values are LSmeans ± s.e. Sample sizes are also provided.

Site	LS mean	±SE LS mean	Sample size
Mt Wellington	754.1	21.69	49
Hobart	856.4	23.76	42
Clarence Lagoon	657.8	22.05	48
Orford	920.6	30.35	25

The only LS mean clutch mass estimates that are not significantly different are those from Orford and Hobart ($P = 0.098$). All other combinations differ significantly ($P < 0.01$).

Finally, in relation to clutch characteristics, relative clutch mass (RCM) was examined. Again, at each site the requirement of homogeneity of slopes was not violated (Mt Wellington, $F_{2,44} = 1.87$, $P = 0.17$; Hobart, $F_{2,36} = 0.27$, $P = 0.76$; Clarence Lagoon, $F_{2,40} = 0.69$, $P = 0.51$; Orford, $F_{2,21} = 0.35$, $P = 0.56$). However, RCM was influenced by maternal SVL at only the Mt Wellington site ($F_{1,46} = 13.55$, $P < 0.001$) (common slope = 0.007). At all other sites the slope of the regression was not significantly different from zero (Hobart, $F_{1,38} = 3.75$, $P = 0.06$; Clarence Lagoon, $F_{1,42} = 2.32$, $P = 0.13$; Orford, $F_{1,22} = 0.17$, $P = 0.69$). Also variation in RCM was detected at only the Mt Wellington site over the three years the site was studied ($F_{1,46} = 10.05$, $P < 0.001$). At all other sites no annual variation was detected (Hobart, $F_{1,38} = 2.24$, $P = 0.12$; Clarence Lagoon, $F_{1,42} = 1.70$ $P = 0.19$; Orford, $F_{1,22} = 1.64$, $P = 0.21$). Again RCM is significantly greater during the 1998-99 season than during the other two seasons of collection ($P < 0.05$). Least square means of RCM (adjusted for maternal SVL) from the above ANCOVAs are presented in Table 6.6.

Table 6.6 Least-square means of RCM for animals from Mt Wellington, Hobart, Clarence Lagoon and Orford during the 1996-97, 1997-98 and 1998-99 field seasons calculated from the above ANCOVAs. Values are LS means ± s.e. Sample sizes are provided in parentheses.

Year	Mt Wellington	Hobart	Clarence Lagoon	Orford
1996-97	0.22 ± 0.014	0.33 ± 0.017	0.24 ± 0.013	NA
	(13)	(14)	(10)	
1997-98	0.25 ± 0.017	0.32 ± 0.019	0.26 ± 0.011	0.32 ± 0.016
	(9)	(12)	(15)	(12)
1998-99	0.29 ± 0.009	0.28 ± 0.017	0.24 ± 0.009	0.29 ± 0.015
	(28)	(16)	(21)	(13)

Again, all RCM data were pooled within sites to examine variation on a geographic scale. The assumption of ANCOVA of homogeneity of slopes was not violated ($F_{3,155} = 0.43$, $P = 0.73$) and there was a significant relationship between maternal SVL and RCM ($F_{3,158} = 20.47$, $P < 0.001$); however, the slope of the relationship was very low (common slope = 0.006). RCM differed between sites ($F_{3,158} = 14.42$, $P < 0.001$) with all combinations of sites being significantly different at the 0.05 level except that involving the Hobart and Orford populations ($P = 0.37$). Least-Square mean clutch sizes from this analysis for each site are presented in Table 6.7.

Table 6.7 Least-square means of RCM for animals from Mt Wellington, Hobart, Clarence Lagoon and Orford derived from ANCOVA using maternal SVL as the covariate. Values are LS means ± s.e. Sample sizes are also provided.

Site	LS mean	SE LS mean	Number
Mt Wellington	0.27	0.008	50
Hobart	0.31	0.009	42
Clarence Lagoon	0.24	0.008	46
Orford	0.30	0.011	25

6.3.3 Offspring characteristics

6.3.3.1 Sex characteristics

Young were sexed during all years of the study. Data were accumulated within sites. At each site the observed sex ratio did not differ significantly from an expected ratio of 1:1 (Clarence Lagoon, $P = 0.864(N = 136)$; Hobart, $P = 0.252(N = 129)$; Mt Wellington, $P = 0.458(N = 147)$; Orford, $P = 0.819(N = 76)$). All offspring characteristics were compared between sexes at each site using two-sample t-tests. Due to the fact that a large number of tests were used, the α value denoting significance was set at 0.01. This ensured that significant differences did not result from chance alone. No sexual variation was detected within sites for any of the characteristics examined. Consequently all subsequent analysis of offspring characteristics was performed on pooled sex data.

6.3.3.2 Annual variation in offspring phenotype

Table 6.8 displays data on offspring morphology and condition for each year of the study and from each study location. Annual variation in each character at each site was examined using ANOVA and the results are presented in Table 6.9.

Table 6.8 Offspring characteristics of *Niveoscincus metallicus* from Mt Wellington, Clarence Lagoon, Hobart and Orford during the 1996-97, 1997-98 and 1998-99 field seasons. All values are means ± s.e. Sample sizes are indicated in parentheses. Condition of offspring is mass divided by SVL; shape provides a measure of relative tail length and is calculated as total length divided by SVL. Fat body mass represents the combined mass of both abdominal fat bodies.

	SVL (mm)				Total length (mm)				Mass (mg)			
Season	**Mt Well**	**Clarence**	**Hobart**	**Orford**	**Mt Well**	**Clarence**	**Hobart**	**Orford**	**Mt Well**	**Clarence**	**Hobart**	**Orford**
1996-97	22.0±0.11 (43)	22.1±0.16 (32)	21.9±0.12 (51)	na	47.8±0.31 (43)	47.4±0.47 (31)	47.4±0.37 (51)	na	219.3±4.1 (43)	213.9±3.9 (32)	212.5±3.6 (51)	na
1997-98	22.3±0.11 (27)	22.4±0.11 (52)	22.4±0.10 (54)	23.1±0.10 (51)	47.8±0.29 (27)	47.9±0.35 (51)	49.5±0.27 (54)	53.1±0.26 (49)	209.1±3.4 (27)	210.7±3.1 (52)	218.4±3.3 (54)	243.9±3.1 (51)
1998-99	22.1±0.13 (101)	22.5 ±0.12 (71)	22.1±0.12 (49)	22.9±0.15 (46)	48.9±0.29 (102)	47.9±0.33 (71)	48.2±0.33 (49)	52.2±0.52 (46)	220.7±3.0 (102)	216.4±3.4 (71)	211.9±4.2 (49)	244.9±5.5 (46)

	Condition (mg/mm)				Shape (Total/SVL)				Fat Bodies (mg)			
Season	**Mt Well**	**Clarence**	**Hobart**	**Orford**	**Mt Well**	**Clarence**	**Hobart**	**Orford**	**Mt Well**	**Clarence**	**Hobart**	**Orford**
1996-97	10.0±0.16 (43)	9.7±0.14 (32)	9.7±0.12 (51)	na	2.17±0.01 (43)	2.15±0.01 (31)	2.16±0.01 (51)	na	2.3±0.15 (43)	2.1±0.14 (32)	1.0±0.08 (51)	na
1997-98	9.4±0.14 (27)	9.4±0.11 (52)	9.7±0.12 (54)	10.6±0.10 (49)	2.14±0.01 (27)	2.14±0.01 (51)	2.21±0.01 (54)	2.30±0.01 (49)	2.5±0.16 (27)	2.4±0.15 (52)	1.0±0.07 (54)	1.4±0.09 (51)
1998-99	10.0±0.12 (101)	9.6±0.11 (71)	9.6±0.16 (49)	10.7±0.20 (46)	2.21±0.01 (101)	2.13±0.01 (71)	2.18±0.01 (49)	2.28±0.01 (46)	2.5±0.10 (79)	2.7±0.11 (53)	1.4±0.16 (25)	2.4±0.25 (26)

Table 6.9 Summary results of statistical tests examining annual variation in offspring characteristics for *N. metallicus* from Mt Wellington, Hobart, Clarence Lagoon and Orford.

Trait	Mt Wellington		Clarence Lagoon		Hobart		Orford	
SVL	$F_{2,168} = 0.67$	$P = 0.51$	$F_{2,152} = 2.15$	$P = 0.12$	$F_{2,151} = 4.18$	$P = 0.02$	$F_{2,95} = 1.49$	$P = 0.22$
Mass	$F_{2,169} = 1.82$	$P = 0.16$	$F_{2,152} = 0.76$	$P = 0.47$	$F_{2,151} = 0.95$	$P = 0.39$	$F_{2,95} = 0.03$	$P = 0.87$
Length	$F_{2,169} = 3.87$	$P = 0.02$	$F_{2,150} = 0.42$	$P = 0.66$	$F_{2,151} = 10.9$	$P < \mathbf{0.01}$	$F_{2,93} = 2.39$	$P = 0.13$
Condition	$F_{2,168} = 3.53$	$P = 0.03$	$F_{2,152} = 1.17$	$P = 0.31$	$F_{2,151} = 0.50$	$P = 0.60$	$F_{2,93} = 0.04$	$P = 0.85$
Shape	$F_{2,168} = 12.9$	$P < \mathbf{0.01}$	$F_{2,150} = 1.45$	$P = 0.24$	$F_{2,151} = 9.24$	$P < \mathbf{0.01}$	$F_{2,93} = 1.31$	$P = 0.25$
Fat Body	$F_{2,146} = 1.05$	$P = 0.35$	$F_{2,134} = 3.46$	$P = 0.03$	$F_{2,127} = 3.18$	$P = 0.05$	$F_{2,75} = 18.2$	$P < \mathbf{0.01}$

Annual variation was generally not common in any of the characteristics examined. No significant differences were detected in either SVL or mass within any population. At the Hobart site, total length was greater in animals born during the 1997-98 season than in those born during either the 1996-97 or 1998-99 seasons. Subsequently body shape (total length/SVL) also differed significantly at the Hobart site in an identical fashion to the pattern observed for total length. Body shape differed significantly at the Mt Wellington population with offspring born during the 1998-99 season having a greater relative body shape (i.e. longer total length for any given SVL) than did those born during both previous season. The only other interannual variation observed was at Orford, where offspring born during the 1998-99 season had significantly greater fat deposits than those born during the 1997-98 season.

6.3.3.3 Geographic variation in offspring phenotype

Due to the fact that inter-annual variation in the measured phenotypic characteristics was low and because mothers were held under identical laboratory conditions during each season, data were pooled to examine inter-site variation. Initially mass and SVL at birth were analysed using ANCOVA with maternal SVL (mm), mass (mg), inter-limb length (mm) and fat reserves (mg) acting independently as covariates. No effect of any maternal characteristic on offspring SLV or mass was observed (Table 6.10). Consequently, subsequent tests on offspring characteristics were conducted using ANOVA. All six offspring characteristics varied significantly on a geographic scale (SVL, $F_{3,573} = 21.0$, $P < 0.01$; total length, $F_{3,570} = 79.7$, $P < 0.01$; mass, $F_{3,574} = 30.0$, $P < 0.01$; condition, $F_{3,571} = 27.2$, $P < 0.01$; body shape, $F_{3,569} = 102.9$, $P < 0.01$; fat bodies, $F_{3,489} = 70.0$, $P < 0.01$) (Table 6.11).

Table 6.10 Effect of maternal characteristics (covariate) on offspring size and mass in *N. metallicus*. Data summarise ANCOVA results examining geographic variation in offspring phenotype. In all cases the requirement of homogeneity of slopes was not violated.

Maternal effect	Offspring mass (mg)		Offspring SVL (mm)	
	F	*P*	*F*	*P*
SVL (mm)	$F_{1,155} = 1.35$	0.25	$F_{1,155} = 1.85$	0.18
Mass (mg)	$F_{1,155} = 3.54$	0.06	$F_{1,155} = 2.66$	0.11
Inter-limb length	$F_{1,155} = 1.52$	0.20	$F_{1,155} = 1.85$	0.18
Fat reserves	$F_{1,155} = 0.01$	0.92	$F_{1,155} = 0.01$	0.92

Table 6.11 Offspring characteristics of *Niveoscincus metallicus* from Mt Wellington, Clarence Lagoon, Hobart and Orford. All values are means ± s.e. Sample sizes are indicated in parentheses. Condition of offspring is mass divided by SVL and shape provides a measure of relative tail length, calculated as total length divided by SVL. Fat body mass represents the combined mass of both abdominal fat bodies.

Site	SVL (mm)	Total length (mm)	Mass (mg)	Condition (mg/mm)	Body shape (Total/SVL)	Fat bodies (mg)
Mt Wellington	22.4±0.07	47.8±0.21	214.0±2.04	9.5±0.07	2.1±0.005	2.4±0.08
	(155)	(153)	(155)	(155)	(153)	(137)
Clarence	22.1±0.08	48.4±0.20	218.5±2.17	9.9±0.08	2.2±0.005	2.4±0.07
	(171)	(172)	(172)	(171)	(171)	(149)
Hobart	22.2±0.07	48.4±0.20	214.4±2.16	9.6±0.08	2.2±0.005	1.1±0.06
	(154)	(154)	(154)	(154)	(154)	(130)
Orford	23.0±0.09	52.7±0.29	244.4±3.08	10.6±0.11	2.3±0.00/8	1.7±0.11
	(97)	(95)	(97)	(95)	(95)	(77)

The clear trend in the data is that animals from the Orford site are both longer (SVL and total) and heavier, and have a greater relative body length and condition than neonates from all other sites. Offspring from Mt Wellington also had a greater SVL than those from Clarence Lagoon; however, this difference was not as large as that observed between Orford and any other site. Animals from Mt Wellington also had a lower level of condition (mg/SVL) than did animals from Clarence Lagoon. However, again this difference is less than that evident between Orford animals and all other populations. Body shape (total length/SVL) also varied between other sites. Clarence Lagoon and Hobart offspring had a greater relative shape than those from Mt Wellington. Again, however, these differences were somewhat smaller than those between Orford and any other site. Fat body mass was the only characteristic to vary in a unique manner. The only sites not to differ were the two high altitude sites. Both low altitude sites had somewhat smaller fat reserves in the

abdomen of offspring; however, animals from Orford carried more reserves than those from Hobart.

6.4 Discussion

6.4.1 Adult body size

There was little variation in adult body size in *N. metallicus*. No inter-annual variation within populations was evident for males, although such variation was observed for females at the Clarence Lagoon and Hobart sites. This variation was, however, quite small and probably reflected collection bias. Similarly differences between males and females at the Hobart site may reflect a bias towards the collections of larger males; smaller very cryptic males were difficult to see at this structurally complex site, while pregnant females of all sizes basked more overtly. In contrast between-site variation in mean SVL was evident in both males and females. Generally, animals from the low altitude sites (Hobart and Orford) were slightly smaller than lizards from the high altitude sites (Mt Wellington and Clarence Lagoon). For females this difference was significant only at Hobart, while in males the difference was most noticeable in Orford animals. When SVL distributions are examined, it becomes apparent that animals from high altitude sites are strongly represented by larger animals while low altitude sites are composed of animals covering a much broader size range.

These differences in adult body size are, however, quite small in comparison to that observed in other closely related species which occur over large geographic ranges. Wapstra and Swain (in press) showed that *Niveoscincus ocellatus* populations displayed substantial differences in adult body size over a similar altitudinal range to that investigated for *N. metallicus*. Low altitude *N. ocellatus* (from Orford) had a mean SVL of 62.7 mm while the value for high altitude populations (Central Plateau) was 72.5 mm. Likewise variation in mean SVL in populations of *Niveoscincus pretiosus*, another widely distributed snow skink, can be similar to that reported in *N. ocellatus* (personal observation). Thus maximum differences in mean SVL of 2 to 3 mm between populations of *N. metallicus*, while significant, most probably simply reflect variation in growth histories resulting from proximate environmental effects rather than any variation in population genotype. Indeed the fact that all populations appear to give birth at a similar

minimum SVL (47 to 48 mm) and reach a similar maximum size (approximately 64 to 65 mm) supports this conclusion. No distinct size classes were evident from SVL distributions for any of the sites examined. Thus animals cannot be assigned to age cohorts based on SVL alone. Chapter 7 examines age-size relationships in female *N. metallicus* in further detail using the technique of skeletochronology. Consequently the remainder of this discussion on adult body size will focus on the effects of maternal SVL on various reproductive characteristics.

6.4.2 Clutch and offspring variation

Variation in clutch and offspring characteristics was examined on both an annual and geographic basis. Inter-annual variation within a population can only result from phenotypic plasticity in the face of some form of environmental variability. Thus the quality of an environment that a female experiences can affect fecundity and offspring quality (Bernado, 1991; Niewiarowski and Roosenburg, 1993; Shine and Harlow, 1993; Sorci *et al.*, 1996; Smith, 1998; Schneider *et al.*, 1999). In contrast, geographic variation in life history can reflect both proximate environmental influences and evolved variation in genotype, or a combination of these two factors (Smith *et al.*, 1995). To minimise the effects of phylogenetic history, all sites employed in this study were chosen on the basis of minimum divergence between populations (Chapter 3). However, any geographic variation in life history may still have a substantial genetic component.

6.4.2.1 Maternal affects

All litter characteristics examined in *N. metallicus* (litter size, mass and RCM) were correlated with maternal SVL. In contrast, offspring mass was independent of maternal size. Maternal effects have been reported in a number of lizard and snake species for a variety of reproductive variables, including the initial decision to reproduce, clutch size, clutch mass, RCM and offspring size (mass) (Dunham and Miles, 1985; Seigel and Ford, 1987; Dunham *et al.*, 1988; Schwarzkopf, 1992; Taylor *et al.*, 1992; Forsman and Shine, 1995; Madsen and Shine, 1996; Bauwens and Diaz-Uriarte, 1997; Olsson and Shine, 1997; Rohr, 1997; Smith, 1998; Abell, 1999; Swain and Jones; 2000b; Wapstra and Swain, in press). However, some studies have indicated that maternal SVL does not influence reproductive characteristics, especially clutch size and RCM, in some species (Goldberg,

1974; Stewart, 1979; Guillette and Casas-Andreu, 1987; Dunham *et al.*, 1988; James, 1991; Howland, 1992; Schwarzkopf, 1992; Pamula, 1997). The size of a female can obviously influence the amount of space available for young (Forsman and Shine, 1995; Olsson and Shine, 1997). Thus, bigger females may be able to produce more offspring and an overall heavier clutch. Female size also influences the amount of fat that can be stored for reproduction (Whittier and Crews, 1990; Pamula, 1997). A positive relationship between clutch mass and size and maternal SVL has also been reported in the sister species *N. ocellatus* (Wapstra and Swain, in press) and has also previously been reported in this species (Jones and Swain, 1996).

6.4.2.2 Inter-annual variation

Annual variation in clutch size was evident at only one of the study sites. In the 1996-97 season, females from Hobart produced larger litters for any given SVL than in subsequent years. Females from Clarence Lagoon also showed a degree of annual variation with smaller litters during the 1998-99 season, that approached significance. Although the evidence is not strong, it does appear that *N. metallicus* is able to adjust this life-history characteristic in response to proximate environmental variations. Of interest to this discussion is the fact that, during the 1996-97 season, Hobart offspring were significantly shorter (total length) and tended towards a smaller SVL than in subsequent seasons, but no variation in mass occurred. No annual variation in any of these birth traits was detected in Clarence Lagoon offspring or newborns from Orford or Mt Wellington.

Most theoretical treatments of offspring size versus number suggest that litter size should be more variable than offspring size (specifically mass) on an annual basis (Smith and Fretwell, 1974; LaLonde, 1991; Forsman and Shine, 1995). However, in species where litter size is low this prediction does not always hold. A number of previous studies of viviparous reptiles have demonstrated annual variation in either litter size (Parker and Pianka, 1975; Reznick and Sexton, 1986; Seigel and Fitch, 1985; Smith *et al.*, 1995) or offspring size (Andren and Nilson, 1983; Bauwens and Verheyen, 1987; Scwarzkopf, 1992; Wapstra and Swain, in press). For example Schwarzkopf (1992) found no significant annual variation in litter size in *Eulamprus tympanum*, but significant variation in offspring size was detected. Forsman and Shine (1995) reported that both litter size and offspring size were equally variable in *Lampropholis delicata* across a large geographic

range, while Wapstra and Swain (in press) found annual differences in offspring size in *N. ocellatus*, but no concomitant variation in litter size. Clearly, *N. metallicus* shows different responses to its sister species, *N. ocellatus*. Annual variation in litter size in *N. metallicus* was apparent at one site and strongly indicated at another, while birth mass did not vary at any of the study sites and there was only small variation in length within the Hobart population. Thus *N. metallicus* appears to conform to theoretical predictions. Swain and Jones (2000b) examined the effects of maternal environment (thermal opportunity and nutrition) during gestation in *N. metallicus* and found that poor nutrition and restricted access to heat during gestation had no effect on litter size, but did influence offspring mass. Thus my observations suggest that any variation in litter size must be a result of proximate variation prior to ovulation resulting in differential recruitment of follicles. Lack of annual variation in offspring mass in natural populations may thus indicate that females at all sites can collect enough resources to maintain this characteristic. Consequently, offspring mass is perhaps more strongly influenced by proximate effects during gestation itself, when maternal food resources are generally available in large quantities, than during earlier collection of resources, when food levels and/or resources may be less. Thus, the combined results of my study and that by Swain and Jones (2000b) suggest that proximate environmental effects influence both clutch size and offspring mass, but at different times. Similarly Doughty and Shine (1998) demonstrated that energetic reserves during vitellogenesis were the primary determinate of clutch size in the skink *Eulamprus tympanum*. In the related species *N. ocellatus*, Wapstra (2000) also found that offspring were significantly affected by thermal opportunity during gestation, while clutch size was not.

The slope (0.197) of the common regression relating maternal SVL and litter size indicated that an increase of approximately 5 mm in SVL is required for mothers to produce one additional offspring. This value is greater than that reported for *N. ocellatus* at low altitude (0.109 Orford site) (Wapstra and Swain, in press) and is also larger than that previously described for *N. metallicus* (0.124) (Jones and Swain, 1996). The relatively steep slope that I observed may also help to explain the occurrence of some annual clutch size variation in this species and its absence in *N. ocellatus* (Wapstra and Swain, in press) and a number of other recently studied species. In *N. ocellatus* a female must undergo a substantial increase in SVL in order to produce one extra young. Interestingly high altitude *N. ocellatus* require an increase of about 5 mm for each additional young and display more

variation in litter size than females from a low altitude population (Wapstra and Swain, in press). These observations suggest that in species/populations where large growth increments are required to achieve an increase in litter size, relatively small differences in the energy reserves available for reproduction are most likely to be reflected as variation in offspring size rather than number. In contrast, when an increase in offspring number is associated with a relatively small increase in maternal SVL, it may be simplest to reduce clutch size in poor years and maintain offspring size at a fairly constant value.

Annual variation was also detected in clutch mass and RCM, but at one site only (Mt Wellington) and only in one season (1998-99). Nevertheless, this result clearly shows that proximate factors can influence reproductive output in *N. metallicus*. ANCOVA also indicated that there was a relationship between maternal SVL and clutch mass at each site: larger females have heavier litters (more young of a similar size). This would suggest that larger females have more energy to channel into clutch production (extra young). RCM did not vary with maternal SVL at three of the four study sites, and was only slightly affected by maternal SVL at the Mt Wellington site (slope = 0.007). Thus very much large females produce clutches with a similar RCM to smaller females. Variation on an annual basis in both of these reproductive characteristics is common in many squamates (Shine, 1980; Schwarzkopf, 1992; Olsson and Shine, 1997; Wapstra and Swain, in press). Schwarzkopf (1992) found that RCM did not vary with year, age or size of mothers in *Eulamprus tympanum* over a two year period. Similarly Wapstra and Swain (in press) found only minimal annual variation in RCM in *N. ocellatus*. The observation that relative clutch mass varies much less than clutch size may indicate that proximate effects act at different times on these reproductive characteristics. Swain and Jones (2000b) reported that RCM in *N. metallicus* was dramatically reduced when animals were held under poor conditions in the laboratory during gestation. Thus, as with offspring mass, and indeed perhaps due to offspring mass variation, RCM may be more significantly influenced by conditions during gestation than those experienced before ovulation.

Previously, Swain and Jones (2000a) have demonstrated the presence of facultative placentotrophy in *N. metallicus*. Facultative placentotrphy can be simply defined. It is the supplemental transfer of nutrients in excess of the minimum requirements for successful development of young (Stewart, 1989). It has been demonstrated in a number of species by comparing the chemical composition of eggs and neonates (Thompson *et al*,

1999a,b,c). To date the most convincing evidence of facultative placentotrophy is the demonstration that larger females give birth to larger offspring, but do not ovulate larger eggs (Stewart, 1989; Thompson *et al.*, 1999a,b,c; Swain and Jones, 2000a). This has been previously demonstrated in *N. metallicus* (Swain and Jones, 2000a). In this study large females were not observed to give birth to significantly larger young, although there was a trend (Table 6.10) for heavier females to give birth to heavier young. The lack of annual variation in offspring mass at any site may indicate that *N. metallicus* is able to use facultative placentotrophy to maintain offspring mass at a constant size from year to year. Consequently, the fact that offspring mass and size vary significantly on an annual basis in the sister species *N. ocellatus* (Wapstra and Swain, in press) may indicate that in this species facultative placentotrophy is less important or absent and that reserves collected during vitollegenesis, when food availability may be more variable, has a greater impact on offspring mass.

6.4.2.3 Geographic variation

Significant variation in clutch size, mass and RCM was observed on a geographic scale. While in all cases these variables were influences by maternal SVL, there was never any variation in the slope of the relationship, indicating that at all sites maternal effects were similar. Clutch size and mass varied significantly between sites with high altitude mothers tending to have smaller or lighter clutches. High altitude females also had lower RCMs than their low altitude con-specifics. Maximum clutch size also varied in a similar manner. Maximum clutch size is generally 6 young at Orford and Mt Wellington; however, clutches of up to 8 offspring have been recorded at the Hobart site and clutch sizes never exceeded 5 at Clarence Lagoon in my samples. In contrast to these findings, offspring mass varied in a very different way. Young from Orford were significantly larger (mass, SVL, total length, condition and shape) than were young from all other sites. Young from all other sites were generally quite similar in body characteristics. These results are in contrast to results found for a number of other species occupying wide geographic ranges. Sinervo (1990b) found that high elevation oviparous populations of *Sceloporus undulatus* produced more young, which were smaller than those from low altitude. Wapstra and Swain (in press) found that high elevation *N. ocellatus* (viviparous) populations produced more young, which were larger than their low altitude con-specifics, even with maternal SVL factored out. Interestingly, they also found that high altitude females had a similar

RCM to low altitude populations, a pattern that is quite different to that observed in *N. metallicus*. Forsman and Shine (1995) also found that geographically widespread oviparous populations of *Lampropholis delicata* displayed significantly larger levels of fecundity in southern populations (colder sites). Again this trend was not a simple consequence of variation in maternal body size. Taylor *et al.* (1992) also indicated that clutch size differed between high and low altitude oviparous sub-species of *Cnemidophorus tigris*, independent of maternal SVL variation, with high altitude populations having larger clutches.

In contrast to these results Rohr (1997) found that high altitude populations of the viviparous water skink *Eulamprus tympanum* had lower size specific reproductive output (RCM) than did low altitude animals. Rohr concluded that this result was a consequence of greater resource limitation at high altitude. His findings are similar to those observed in *N. metallicus* in which high altitude populations have lower RCMs and litter masses and a tendency towards smaller litter sizes. Variation in reproductive output, independent of maternal size effects, has been attributed to variation in resource availability (Vitt and Congdon, 1978; Dunham, 1982; Vitt and Price, 1982; Shine, 1992; Rohr, 1997), but a heritable component has been identified (Mateo and Castanet, 1994). Swain and Jones (2000b) found that RCM was affected by low food availability during gestation and was not influenced by decreased thermal opportunity, although the decrease in RCM was a direct result of reduced offspring mass, a factor that failed to vary between natural populations in my investigation. Similarly Wapstra (2000) found no decrease in RCM in *N. ocellatus* exposed to low thermal opportunity. Food is abundant at high altitude in Tasmania during summer months (personal observation) and low food availability is unlikely to represent a major problem in most years (although females do have less time to collect food). The possibility exists therefore that variation in reproductive output (RCM) may in part represent some evolved adaptation to high altitude. High altitude females live significantly longer than their low altitude con-specifics (Chapter 7) and reduced annual output may be correlated with increased future reproductive potential. In contrast low altitude animals have a low life expectancy, and an increase in current reproductive effort may represent a significant adaptation to increased predation and competition pressures (Adolph and Porter, 1996). Indeed Chapple (2000) has shown that tail loss in *N. metallicus* is more common at low altitude than at high altitude and tail breaks are more likely to be

near the base of the tail at low altitude suggesting either that predation risk is greater at low altitude or that survival of high risk encounters is greater.

Niveoscincus metallicus may therefore display some level of evolutionary adaptation in RCM to high altitude, associated with slower growth, decreased predation and increased life span. However, *N. metallicus* occurs sympatrically with the alpine restricted *N. microlepidotus* and *N. greeni* at the upper extent of its range. At these sites the alpine species display biennial reproductive cycles, while *N. metallicus* retains an annual cycle in which 100% of females normally reproduce each year. Thus, despite the fact that this species does decrease reproductive investment at high altitude, it has been unable to take an additional step to a biennial cycle. Melville and Swain (2000b) identified high altitude *Niveoscincus* species as a separate group in the evolutionary history of the genus. This clade consisted of *N. microlepidotus*, *N. orocryptus*, *N. greeni* and *N. ocellatus*. Of these animals, the three high altitude restricted species appear to be biennial breeders (Greer, 1982; Hutchinson *et al*, 1989). Thus this mode of reproduction appears to have evolved in a different group to that containing *N. metallicus* and this species may be excluded from extreme altitudes by an inability to collect sufficient resources to permit annual breeding.

Data collected to date for *N. metallicus* (Swain and Jones, 1994; Jones and Swain, 1996; this study) and *N. ocellatus* (Wapstra and Swain, in press) clearly demonstrate that many life-history characteristics differ between the two species, despite their obvious close relationship. Recent phylogenetic studies by Melville and Swain (1998; 2000b) describe the evolutionary history of the entire *Niveoscincus* group. These authors have concluded that *N. metallicus* evolved from a low altitude species and split from other members of the genus very early in their evolutionary history (Melville and Swain, 2000b) and subsequently colonized high altitude environments. In contrast *N. ocellatus* appears to have evolved from a high altitude species (Figure 3.1) and to have subsequently colonized low altitude habitats. This very different history may explain the variation in life history patterns exhibited by the two species, most notably the differences in adaptation of growth patterns, adult size, parental investment and offspring characteristics.

6.4.2.4 Offspring characteristics

While both RCM and litter mass are relatively high at the low altitude sites (Hobart and Orford) the partitioning of input between offspring is very different. At Hobart more small offspring are produced, while at Orford fewer large young are the norm. Although the evolution of offspring size and offspring size/litter size trade offs has been examined (Smith and Fretwell, 1974; Roff, 1992; Schwarzkopf, 1992; Stearns, 1992; Olsson and Shine, 1997; Abell, 1999), factors that affect offspring size, and its variation, in reptiles are not fully understood (Wapstra and Swain, in press). However, variation in offspring size on a geographic scale is generally interpreted as adaptive, since fitness is highest in animals that produce the most offspring that survive to reproduction (Shine and Schwarzkopf, 1992; Sinervo and Doughty, 1996). Thus, selection for neonate size (Vitt and Price, 1982) coupled with the amount of energy available for reproduction (Sinervo and Doughty, 1996) must be key determinates of litter size. The evolution of offspring size and number is therefore controlled by the trade-off between fecundity advantages arising from production of many small young and the enhanced survival potential associated with the production of larger young (Lloyd, 1987; Forsman and Shine, 1995).

In species occurring over wide geographic ranges, larger offspring are often associated with colder climates and high altitudes (Ferguson *et al.*, 1980; Forsman and Shine, 1995; Rohr, 1997 Wapstra and Swain, in press). However, this is not always true (Sinervo, 1990b). Larger size at birth is believed to occur when potential for growth is low or when there is strong selection on offspring survival (Brockelman, 1975; Rowe, 1994; Forsman and Shine, 1995; Wapstra and Swain, in press). In *N. metallicus* high altitude populations produced young that were the same size as those from Hobart and much smaller than those from Orford. Thus, reduced potential for growth, which is evident at high altitude, does not appear to be acting as a major selection force on offspring size, as it appears to do in *N. ocellatus*. In contrast, selection for offspring survival may be a reason for increased offspring mass and size at the Orford site. In many studies offspring size increases when food or water availability is high (Ballinger, 1977; Olsson and Shine, 1997; Swain and Jones, 2000b). However, Abell (1999) showed that offspring of *Sceloporus virgatus* increased in size during drought periods. This increase in size was also accompanied by a decrease in litter size. Abell (1999) argued that this response could result from advantages received from increased offspring size during periods of intense among-hatchling

competition for resources (Ferguson *et al.*, 1982) or may represent a by-product of a bet-hedging strategy to produce smaller clutches at times when the probability of clutch failure is high (Nussbaum, 1981). However, if this argument has validity for *N. metallicus* quite different reasoning must apply in the sister species *N. ocellatus* in which young born at the Orford site are significantly smaller than those born at high altitude (Wapstra and Swain, in press).

Another selective force exists, however, which may also account for the observed increase in offspring mass at the Orford site. Numerous invertebrate predators are present at Orford that are either rare or absent at other sites. These include a number of spider species that are able to capture and kill small lizards (less than 250 mg). Thus if mothers can shorten the time young spend at risk to this type of predation, they may significantly enhance their overall reproductive success. Thus, differences in predation pressures may be influencing life history in this species. Schneider *et al.* (1999) showed that habitat type, acting through avian predation pressure in a tropical rainforest, exerted a much greater influence on life history in *Carlia rubrigularis*, a widespread leaf-litter skink, than did genetic divergence between populations that were geographically isolated. This pressure could also explain the smaller size of offspring of *N. ocellatus* at this site relative to high altitude offspring. While these young may be small based on intra-specific comparisons, they are still somewhat larger than *N. metallicus* young. Thus these animals may already be too large at birth for many invertebrate hunters to predate them.

While altitude appeared to have no effect on offspring mass and general morphology, it did influence energy reserves available to neonates at birth. Abdominal fat bodies were significantly larger in high altitude young than in young from either of the low altitude sites. This correlates well with the reduced potential for growth and much shorter period available for food collection prior to hibernation in *N. metallicus* at high altitude. Fat body size was also greater in the Orford population than in the Hobart population suggesting that factors such as predation pressure may also be influencing this offspring characteristic. Mothers may provide young with increased fat reserves at this site to enable growth to occur quickly following birth, thus limiting time that young must spend at a potentially vulnerable size. Nevertheless the fact that high altitude young still possess fat bodies that are significantly larger than those in Orford animals suggests that in this case reduced potential for growth and food collection prior to winter are significantly affecting

offspring survival. Fat reserves in offspring collected over large geographic ranges has not previously been assessed in lizards, so it is difficult to fully interpret the significance of these results, beyond concluding that offspring morphology and resource availability at birth can act independently to assist adaptation to environmental variability.

Facultative placentotrophy may in part also explain the presence of increased fat reserves in offspring from high altitude. Facultative transfer occurs before developmental stage 40 is first reached. Young are approximately 150 mg at this stage but must continue to grow to around 210 mg before birth (Swain and Jones, 1997, 2000a). During this time young must survive on their own reserves. At low altitude where conditions are more predictable, this period may be short, while at high altitude significant delays in birth can occur due to poor weather. Thus facultative placentotrophy may provide a method of increasing fat reserves in high altitude populations, both as a means of providing reserves for growth following birth and as a supply of energy and nutrition during the final stages of gestation.

6.4.3 Summary

The database describing geographic variation in life history traits in skinks is limited and very few underlying patterns are evident (Wapstra and Swain, in press). In *N. metallicus* annual variation in clutch characteristics and offspring characteristics were evident, but were not great. Consequently, in this species, as in many other geographically ubiquitous reptiles, proximate adaptation to environmental variation appears possible. Significant levels of geographic variation were evident between populations of *N. metallicus*. Variation on this scale can reflect both proximate effects and evolutionary adaptation (Stearns, 1989). However, despite some support for genetic life history variation between populations (Niewiarowski and Roosenburg, 1993; Sorci *et al.*, 1996), recent research provides strong support for proximate explanations for differences in life history between populations (Schneider *et al.*, 1999; Sorci and Clobert, 1999; Wapstra and Swain, in press). Geographic variation was evident in a number of life history characteristics including clutch size, mass, RCM, offspring size and offspring fat reserves; however, previous work on *N. metallicus* has indicated that almost all of these factors can vary significantly as a result of proximate effects in this species (Swain and Jones, 2000b; this thesis). Thus the evolution of distinct local genotypes may be unnecessary if phenotypic plasticity is sufficient to induce variation in life history traits to suit environmental

conditions. However, the possibility still exists that consistent selection pressures, from both altitudinal and ecological sources, could act on the genotype of populations, thus resulting in genetic variation in life history characteristics, specifically in influencing factors such as current reproductive effort (total litter mass and RCM).

CHAPTER SEVEN

Age and size relationships in *Niveoscincus metallicus*

7.1 Introduction

Age and size at maturity, together with longevity, are central parameters in general life-history models (James, 1991; Galan, 1996) and all can exercise a marked influence on the demographic strategies of squamate reptile populations (Tinkle *et al.*, 1970; Dunham *et al.*, 1988). Age at maturity, specifically, is a pivotal factor in life history theory as fitness is often more affected by variation in this trait than by any other (Stearns, 1992; Galan, 1996; Rohr, 1997). Species distributed over broad geographic and altitudinal ranges often display extensive variation in life-history traits such as age at maturity, growth, and age-specific schedules of fecundity and survivorship (Niewiarowski, 1994). Differences in size and age at first reproduction have been observed often in intra-specific comparisons (Ballinger, 1979; Howland, 1992; Larsen *et al.*, 1993; Tinkle *et al.*, 1993; Rohr, 1997; Wapstra *et al.*, in press). Variations in these traits have important implications for the life-history patterns of populations because after maturity energy is diverted away from maintenance, growth and storage, and directed towards reproduction (Rohr, 1997). Thus a significant trade-off between age at maturity and adult size is often observed (Shine and Charnov, 1992; Rohr, 1997). As adult size is closely related to future fecundity in many reptile species, early maturity may also reduce future reproductive output (Stearns, 1989; Shine and Schwarzkopf, 1992; Bernardo, 1993; Niewiarowski and Dunham, 1994; Wapstra *et al.*, in press). Thus, there are two broad strategies of maturation in squamate reptiles: "early" versus "late" maturity (James, 1991; Galan, 1996). Each is believed to reflect natural selection favoring successful genotypes under particular environmental conditions (James, 1991).

In order to understand delayed maturation, it is important to balance the selection pressure to mature early against trade-offs with other fitness components (Stearns, 1992). Populations displaying late maturation tend to exhibit larger adult size and greater longevity. In contrast, "early" populations tend towards smaller body size and lower

survival (Tinkle *et al.*, 1970; Schwarzkopf, 1994; Adolph and Porter, 1996). Early reproduction may increase life-time reproductive success if there is no reduction in future reproductive output. However, late maturity, accompanied by larger clutch sizes (reflecting larger adult body size) or increased survival of young and mother, may be a more successful strategy under certain situations (Tinkle *et al.*, 1970; Dunham *et al.*, 1988; Galan, 1996).

Life history characteristics vary widely among species and populations. These differences are often ascribed to genetic differences. However, recent work has identified both genetic and environmental sources as important determinants of intra-specific life-history variation (Ferguson and Brockman, 1980; Niewarowski and Roosenburg, 1993; Bernardo, 1994; Smith *et al.*, 1994a; Niewarowski, 1995). It has become clear that reptilian life histories are often phenotypically plastic, varying in response to many environmental variables (Adolph and Porter, 1993). As a result, numerous studies have examined proximate environmental influences on the expression of reptilian life history traits (Ballinger, 1977, 1979; Dunham, 1978; Porter and Tracy, 1983; Jones and Ballinger, 1987; Sinervo and Adolph, 1989, 1994; Sinervo, 1990a; Adolph and Porter, 1996; Rohr, 1997; Wapstra and Swain, in press). Many of these studies specifically address the role of the thermal environment, which frequently exerts strong proximate effects (Adolph and Porter, 1993; Bernardo, 1994; Wapstra, 2000). Thermal environment (varying with both latitude and altitude) determines the length of the growth season in many temperate ectotherms. High altitude populations are thus subject to curtailed periods of suitable weather both on an annual (Adolph and Porter, 1996) and diurnal (Sinervo and Adolph, 1989; Smith and Ballinger, 1994a; Rohr, 1997; Wapstra *et al.*, in press) basis. Adolph and Porter (1996) argued that the reduction in growth opportunity for high altitude populations was directly responsible for delayed maturity, increased longevity and larger adult body size. To date, data from a number of studies tend to support their model (Tinkle *et al.*, 1970; Bruce and Hairston, 1990; Grant and Dunham, 1990; Galen, 1996; Wapstra *et al.*, in press): However, Rohr (1997) found that *Eulamprus tympanum* delayed maturity at high altitude longer than predicted by the Adolph and Porter (1996) model. Thus to fully understand variability in this phenomenon, data from many more species are required.

Reptiles generally show indeterminate growth, with growth slowing following maturity (Schwatzkopf, 1994). While age and size may be correlated within a population, temporal

and individual variation in growth rate cause adult body size to be a poor predictor of age in many species (Andrews, 1982; Dunham *et al.*, 1988). Growth and age are often studied in reptiles by fitting growth models to mark-recapture data (James, 1991, Hudson, 1997). However, this method is impractical in many small cryptic species of skink, such as *Niveoscincus metallicus*. Alternatively, or in addition to mark-recapture programs, skeletochronology has been used in a number of extant and fossil species of reptile and amphibian to accurately estimate age (Gibbons and McCarthy, 1983, 1984; Leclair and Castanet, 1987; Halliday and Verrell, 1988; Hemelaar, 1988; Ryser, 1988; Montori, 1990; Forester and Lykens, 1991; Platz and Lathrop, 1993; Buffrénil *et al.*, 1994; Rogers and Harvey, 1994; Klinger and Musik, 1995; Wake and Castanet, 1995; Esteban *et al.*, 1998; Waye and Gregory, 1998; Caetano and Leclair, 1999; Driscoll, 1999; Horner *et al.*, 1999; Esteban and Sanchiz, 2000; Miaud *et al.*, 2000; Trenham *et al.*, 2000). More specifically, it has successfully been used in a number of lizard species (Castanet, 1978, Tilley, 1984; Castanet *et al.*, 1993; Hudson, 1997; Rohr, 1997; El Mouden *et al.*, 1999; Wapstra *et al.*, in press). Hudson (1997) compared von Bertalanffy growth curves fitted to both skeletochronological and mark-recapture data in *Pseudemoia pagenstecheri* and found that they were not significantly different from each other. He also found that estimates of age at maturity and longevity were similar for both data sets. Despite this, few studies of lizard life histories have incorporated age data estimated from skeletochronology. Exceptions include Mateo and Castanet (1994), Olsson and Shine (1996), Hudson (1997), Rohr (1997) and Wapstra *et al.*, (in press). For reviews on skeletocronology see Castanet *et al.* (1993) and Hudson (1997).

The main aim of this chapter is to examine size and age at maturity, growth patterns and longevity in *N. metallicus* from the four previously described field sites. Few studies have examined intra-specific variation in age and size at maturity in Australian temperate skinks (but see Hudson, 1997, Rohr, 1997 and Wapstra *et al.*, in prep). Hudson examined age, growth and maturity in a number of southeastern Australian skinks including *N. metallicus*. However, his study of this species was limited to populations at high altitude that were identified in Chapter 3 as being genetically distinct. My study examines geographic and altitudinal variation in growth and age at maturity in female *N. metallicus* from a single genetically distinct subgroup. The chapter sets out to answer a number of specific life-history questions.

1) Does female age or size at maturity differ between populations from differing climatic extremes (i.e. altitudes)?

2) Does possible variation in age at maturity simply reflect decreased access to thermal opportunity at high altitude, or do sub-adult animals grow significantly slower when thermal opportunity rather than actual age is examined?

3) Does longevity of female *N. metallicus* increase with altitude, and if so, how might this influence lifetime reproductive output?

4) To what extent does growth decrease following sexual maturity in female animals, and does this vary with altitude?

7.2 Materials and Methods

7.2.1 Skeletochronological age assessment

Animals were captured as part of a general study of life history adaptation (Chapter 6) from the four previously discussed field sites (Chapter 2). Animals were allowed to give birth in the laboratory and from these animals a subset were then killed. Femurs were removed and stored in 70% ethanol until processed. A total of 118 mature females, covering the complete size range of adult animals from each site, were used in this study.

The principle behind skeletochronological aging is that the seasonal nature of growth is reflected in patterns of bone growth and results in Lines of Arrested Growth (LAGs) which occur during periods of winter inactivity in temperate species (Leclair and Castanet, 1987; Castanet *et al.*, 1993). However, one major problem is often observed when employing this method of age determination. This is the loss of LAGs from early life due to remodeling of bone. In many species examined to date, endosteal remodeling is lowest at a point slightly proximal to the mid-diaphysis, where a single large vascular canal passes obliquely through the cortex of the femur (Hudson, 1997). Endosteal remodeling generally increases distally from this canal. This was also the case in *N. metallicus*. Remodeling of bone tissue can be accounted for by examination of smaller femurs in

which no resorption has occurred. From this it is possible to back calculate the number of LAGs lost to remodeling in older animals (Hemelaar, 1985; Leclair and Castanet, 1987).

Femurs were washed and decalcified in 5% nitric acid for 8 hours before rewashing in tapwater for 24 hours. They were embedded in paraffin wax using standard techniques following alcohol dehydration. Embedded femurs were serially sectioned at 10 μm and mounted on slides. These were subsequently dried and stained using Ehrlich's haematoxylin for 30 minutes followed by three rinses in tapwater for 3 minutes. This technique produced distinct LAG bands throughout the bone section. In almost no case was complete resorption of LAGs observed. Even in animals from high altitude it was generally possible to partially see the inner LAG as only one side of the inner medullary cavity was removed. In many cases it was also possible to observe part of a birth line enclosing paler-stained embryonic bone. Hudson (1997) indicated that in his study of several southeastern Australian species, this tissue was evident in 94% of adult animals. In contrast Wapstra *et al.* (in press) found that in *N. ocellatus* the majority of animals had lost their first LAG and in high altitude populations many also lost their second. However, this species is significantly larger than *N. metallicus* and faster growth during sub-adult periods may increase resorption. In older animals the number of LAGs at the periphery became difficult to establish. Castanet *et al.* (1988) suggested that making conservative estimates, resulting in underestimation of age, was the preferred option. All bones were examined over a four-day period and then again in a second blind trial one month later to determine reliability of earlier estimations. During these examinations total number of LAGs, and diameters of the first 4 LAGs were recorded using an eyepiece graticule.

The age of females (in months) was calculated by counting LAGs (i.e. winters lived by animal) plus the number of months of growth since the last winter and the number of months prior to the first winter. All animals from low altitude sites were assumed to have been born during mid January and all high altitude animals were assumed to have been born during mid February (Jones and Swain, 1996; Chapter 6). Differences between mean age of females from each site was assessed using one-way ANOVAs. Homoscedacity of variances was investigated by visual inspection of plots of group standard deviations versus group means. Normality of the data was evaluated through the inspection of a plot

of residuals versus predicted residual values. Significant interactions were further examined using Tukey's studentized range tests.

7.2.2 Growth curves

I used the relationship between SVL and age to construct growth curves using the von Bertalanffy growth model. Two asymptotic growth models based on linear length measurements are most often used in reptile studies: the von Bertalanffy growth model and the logistic-by-length model (Dunham, 1978; Schoener and Schoener, 1978; Andrews, 1982; Hemelaar, 1988; James, 1991; Adolph and Porter, 1996). Growth curves were constructed entirely from the ages of mature females. Data on small, young individuals may be necessary to distinguish among the two separate models, because the major differences between them occur in smaller size classes (Yamaguchi, 1975; Frazer *et al.*, 1990). The von Bertalanffy model predicts that growth (in length) is maximum in newborn lizards, whereas the logistic-by-length model predicts maximum growth later in life (Andrews, 1982). Hudson (1997) fitted both growth models to data on *Pseudemoia pagenstecheri* and determined that the von Bertalanffy model best described growth. Similarly, mark recapture data from five species of *Ctenotus* skinks was better described by the von Bertalanffy model (James, 1991). Stamps (1995) suggested that growth patterns are conservative in squamates, so that related species should display similar basic growth patterns. Frazer *et al.* (1990) concluded that von Bertalanffy growth curves can be produced from data on larger individuals, provided there is reason to believe *a priori* that the model is appropriate. Therefore the absence of data on juvenile animals should not adversely affect estimates of growth parameters using this method. The general form of the von Bertalanffy equation is:

$$SVL_t = a\,(1\text{-}be^{-kt})$$

Where SVL_t is the body size at time t, a is the asymptotic body size, b is a parameter related to initial body size, and k is the characteristic growth rate. The parameters a, b and k were estimated using nonlinear regression techniques (Andrews, 1982; James, 1991) using SYSTAT version 7.0. Growth trajectories were considered to be different at the 0.05 level if the 95% confidence intervals did not overlap (Dunham, 1978; Schoener and Schoener, 1978; James, 1991; Niewiarowski *et al.*, 1997).

7.2.3 Age and size at maturity

In life history theory, the age at maturity is defined as the age at first parturition or oviposition, and not when other events (such as vitellogenesis, ovulation or mating) first occur (Stearns, 1992). In *N. metallicus* this is a significant point, as mating and vitellogenesis occur approximately 8 months before birth. Therefore, in this study I regard age at maturity as the age of parturition of the first litter. Minimum size and age at maturity was estimated as the size and age of the smallest and youngest female to have given birth from each site. To examine growth to maturity I regressed the size of the youngest reproducing female (estimated from the above non-linear regression) against the time available for growth from birth to capture. Mean offspring size estimated from Chapter 6 was assumed to be constant for all years and was used as the value for the y-intercept. Linear regression models have been shown to be appropriate for describing growth in juvenile animals even to first reproduction in some species (Andrews, 1982; Tilley, 1984; Rohr, 1997; Waspstra, 1998). *Niveoscincus metallicus* from high altitude populations experience reduced growing seasons. In order to adjust annual growth to local conditions I corrected for hibernation period. Observations in the field indicate that low altitude populations emerge during late August while high altitude populations are not seen until late September. Low altitude animals go into torpor in mid May while high altitude populations submerge in late March. Thus it is assumed that low altitude populations have a total of 257 active days during a year while high altitude animals are restricted to only 182 days. From the above periods I subtracted the average number of rain days during the indicated periods for each site (Rohr, 1997), using data provided by the Tasmanian Bureau of Meterology (Chapter 2).

7.3 Results

7.3.1 Age distribution between populations

In all populations, except that from Clarence Lagoon, age distribution in mature females was strongly skewed (Figure 7.1), so that younger females represented the majority of animals captured. Clarence Lagoon females displayed a more evenly distributed age pattern with animals of all middle age groups represented strongly in the collection.

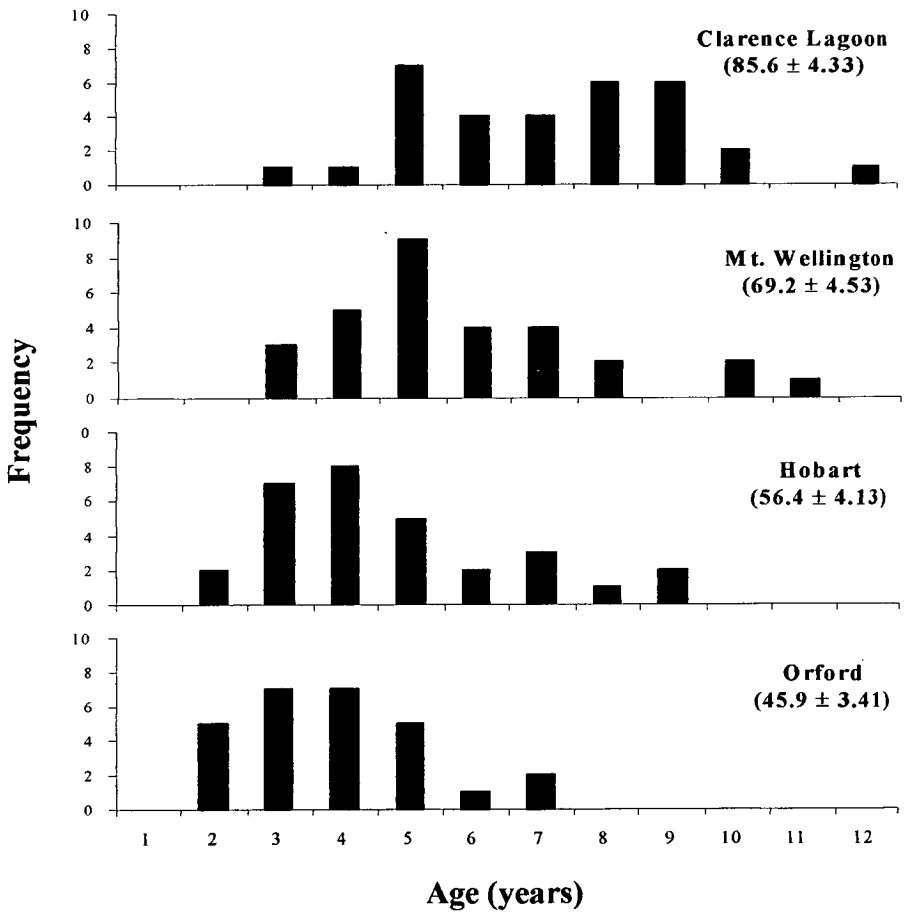

Figure 7.1 Age distribution of female *N. metallicus* from the four study populations, as estimated from the number of LAGs. All LAGs represent one year of growth and females are assumed to be mature at the time of their first parturition. Mean ages (± s.e.) for each population (in months) are also shown.

Mean age differed significantly between sites (ANOVA: $F_{3,115} = 16.83, P < 0.0001$). Females from Clarence Lagoon were generally older than females from all other sites. Hobart and Mt Wellington animals were not significantly different. Orford and Hobart

animals were also not different. Mt Wellington animals were, however, significantly older on average than those from Orford. All comparisons were considered significant at an alpha level of 0.05. Maximum age ranged from 7 years at Orford to 12 years at Clarence Lagoon.

7.3.2 Growth in *N. metallicus*

Table 7.1 presents the parameter values and the upper and lower 95% confidence intervals estimated from the von Bertalanffy growth model. Parameters are described in Section 7.2.2.

Table 7.1 Parameter estimates for von Bertalanffy growth models generated from non-linear regression of size (SVL) and age for female *Niveoscincus metallicus* from Clarence Lagoon, Mt. Wellington, Hobart and Orford. Upper and lower estimates are 95% confidence intervals.

	Parameters from von Bertalanffy growth model									
	a			**b**			**k**			r^2
Site	**lower**	**mean**	**upper**	**lower**	**mean**	**upper**	**lower**	**mean**	**upper**	
Clarence	56.66	**58.02**	59.38	0.578	**0.616**	0.653	0.029	**0.036**	0.044	0.97
Wellington	58.80	**60.86**	62.91	0.598	**0.635**	0.672	0.026	**0.031**	0.036	0.97
Hobart	56.96	**58.69**	60.42	0.581	**0.620**	0.660	0.035	**0.043**	0.051	0.96
Orford	56.56	**58.88**	61.19	0.540	**0.602**	0.665	0.040	**0.054**	0.069	0.93

Both *a* and *b* appear not to differ between sites. This is not surprising as adult body size and initial size at birth do not differ greatly between populations. Of interest are the differences evident in k (characteristic growth rate). Females from low altitude sites appear to grow at a faster rate than females from high altitude. However, differences were only significant between animals from Orford and Mt. Wellington (non-overlap of 95% confidence intervals). Von Bertalanffy growth curves overlaid on age data for each population are shown in Figures 7.2 to 7.5. The growth equation provided a reliable fit to observed data (r^2 values ranging from 0.93 to 0.97). Clearly there is considerable overlap in parameter estimates between sites and differences occur only in the time to asymptote.

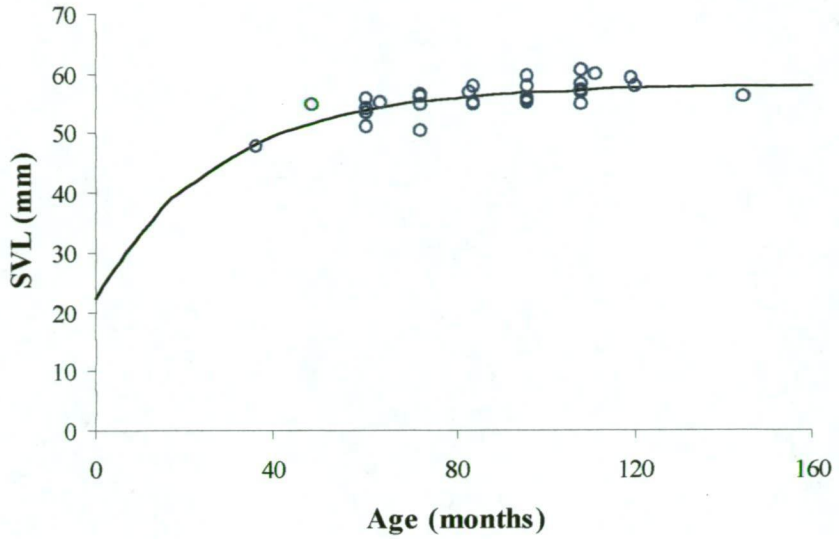

Figure 7.2 Von Bertalanffy growth curve fitted to age versus size data for female *N. metallicus* from Clarence Lagoon. All data points represent mature females carrying young or immediately post-partum. The y-intercept value was calculated from mean juvenile size for young born in the laboratory during 1996-97, 1997-98 and 1998-99 field seasons.

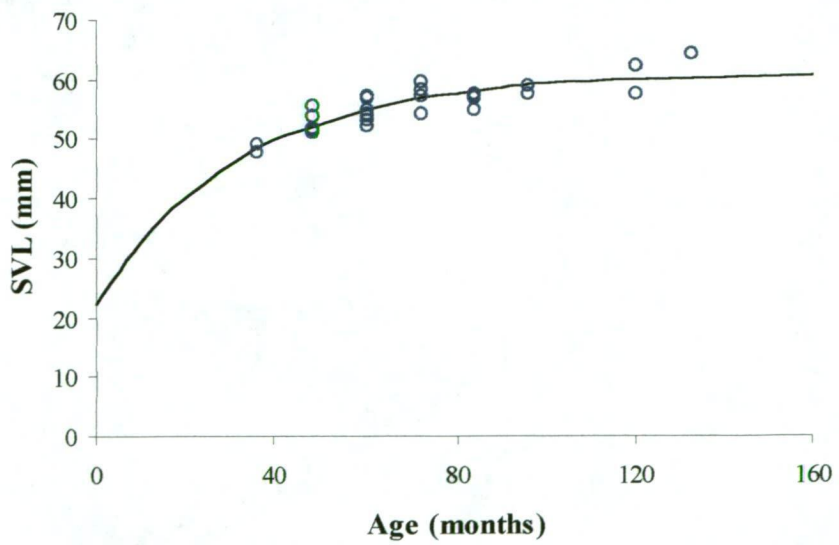

Figure 7.3 Von Bertalanffy growth curve fitted to age versus size data for female *N. metallicus* from Mt Wellington. All data points represent mature females carrying young or immediately post-partum. The y-intercept value was calculated from mean juvenile size for young born in the laboratory during 1996-97, 1997-98 and 1998-99 field seasons.

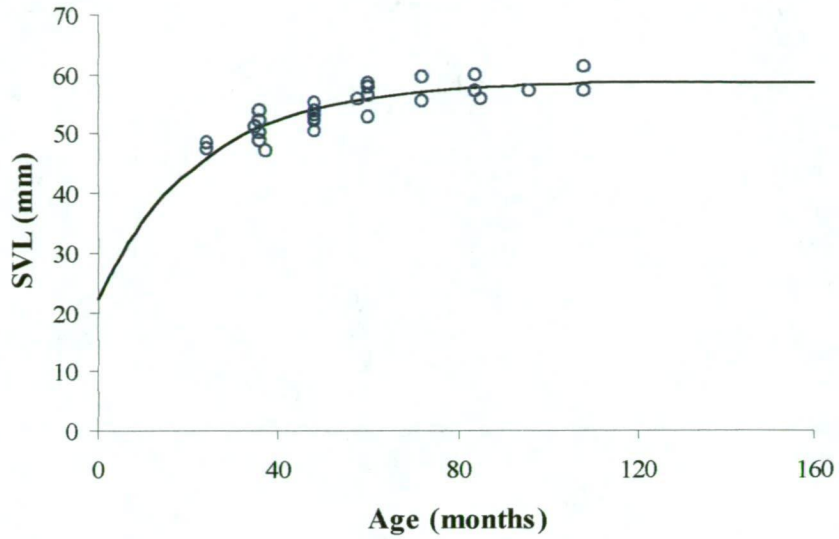

Figure 7.4 Von Bertalanffy growth curve fitted to age versus size data for female *N. metallicus* from Hobart. All data points represent mature females carrying young or immediately post-partum. The y-intercept value was calculated from mean juvenile size for young born in the laboratory during 1996-97, 1997-98 and 1998-99 field seasons.

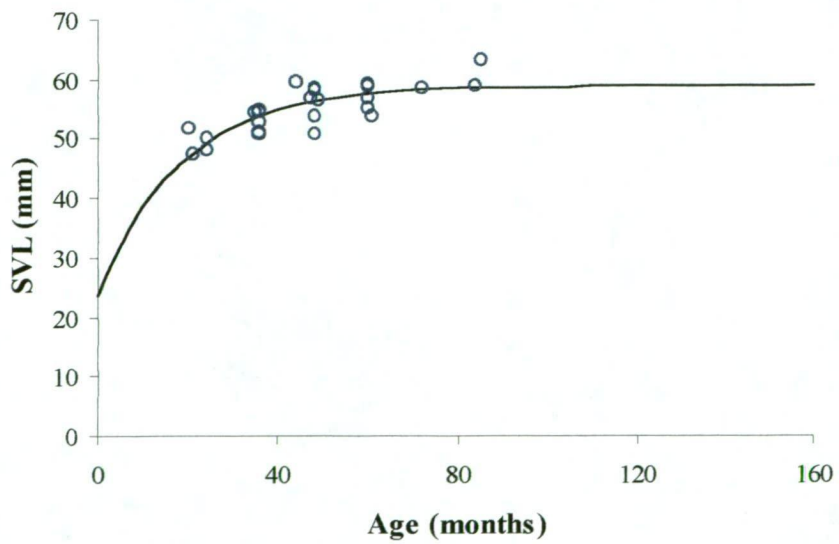

Figure 7.5 Von Bertalanffy growth curve fitted to age versus size data for female *N. metallicus* from Orford. All data points represent mature females carrying young or immediately post-partum. The y-intercept value was calculated from mean juvenile size for young born in the laboratory during 1997-98 and 1998-99 field seasons.

7.3.3 Growth to maturity in *N. metallicus*

Minimum size at maturity was relatively consistent between all populations. The smallest mature female observed at Clarence Lagoon and Mt Wellington were 47.7 mm and 47.9 mm respectively. Orford and Hobart displayed minimum sizes at maturity of 48.1 mm and 47.2 mm (Chapter 6). Animals from both low altitude sites matured in 2 years. This was one year faster than for animals at high altitude sites (Figure 7.1). However, when I account for differences in activity and available thermal opportunity (Figure 7.6) it becomes apparent that there is little difference between growth rates of high and low altitude populations. The slopes of the linear regression lines for animals from Mt Wellington and Clarence Lagoon were 0.099 and 0.104 respectively. Animals from Orford and Hobart displayed decreased slopes of 0.080 and 0.079, indicating that growth was perhaps slower in young from low altitude. However this cannot be tested statistically since each line is based on only two data points. It seems unlikely, however, that this observed difference is real, as animals from high altitude sites are pre-adapted for activity during cool periods (Chapter 5). Young from these sites may therefore be active more often that predicted by these estimates (i.e. during periods of rain, as was observed in the field). If this is so it would decrease the slope of the growth estimates for females from these populations, restoring it to a level similar to that calculated for low altitude animals.

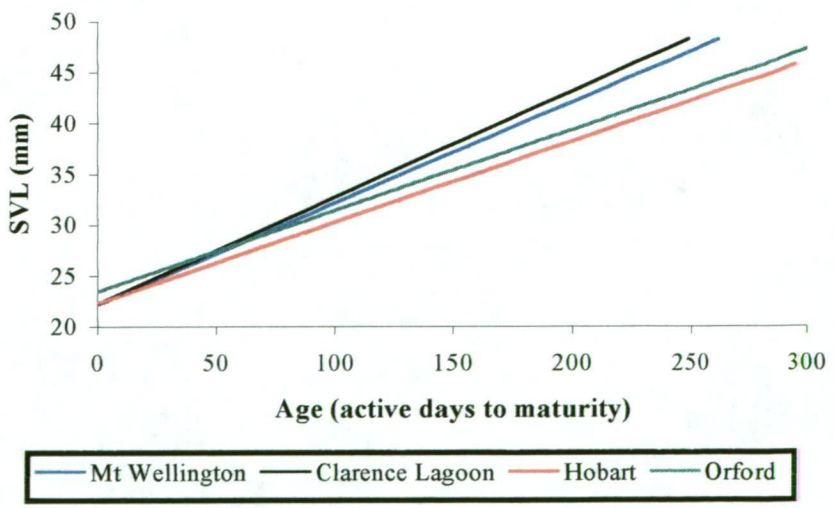

Figure 7.6 Growth to first reproduction for female *N. metallicus* from low and high altitude field sites. The time available for growth was adjusted for differences in the length of activity and for the number of rain days for each site, as in Rohr (1997). Size at maturity was determined from the von Bertalanffy growth models described in Section 7.3.2.

7.4 Discussion

Skeletochronology proved to be an effective method of aging female *N. metallicus*. Femoral sections were clear and easily interpreted and LAGs were clearly visible. Resorption of bone in older animals was usually minimal, with at least part of the early LAGs remaining. A birth line was also evident in the majority of animals from all populations. LAG distribution in mature animals appeared to mirror typical lizard growth patterns with initial pre-maturation LAGs being well spaced and later LAGs being closely packed, indicating decreased growth later in life. This pattern is considered to be due primarily to energy demands placed upon animals for gonadal maturation and other costs associated with reproduction (Andrews, 1982; Anderson and Vitt, 1990; Shine and Schwarkopf, 1992; Schwarkopf, 1994; Rohr, 1997). Closely grouped LAGs in older animals were still easily distinguished and easily counted. If growth ceased in older animals, then additional LAG formation would also cease, resulting in an underestimation of age in older animals. However, Hudson (1997) found that a related species, *Psuedemoia pagenstecheri*, continued to grow even when approaching maximum age.

I examined growth and maturity in female *N. metallicus* only; however, some discussion of possible sexual variation in this species in required. Both female and male animals mature at a similar size and reach a similar maximum size (Chapter 6). There is also no variation in size at birth between sexes (Chapter 6). Wapstra (1998) found no variation in growth rate between sexes in juvenile *N. ocellatus* held in the laboratory. He also found no variation in size at maturity and maximum size between sexes in this species. Likewise Hudson (1997) also found no differences in growth rates of male and female *P. pagenstecheri*, although females reached a slightly larger maximum size. This pattern of increased body size in females is common in skinks (Greer, 1989; James, 1991; Hutchinson, 1993; Forsman and Shine, 1995). However, models of life-history evolution predict a general similarity in growth rates and population responses of closely related species experiencing the same overall environmental conditions (Dunham and Miles, 1985; James, 1991; Vitt and Blackburn, 1991; Stamps, 1995). Thus, given similarities in adult body size between male and female animals, it seems probable that *N. metallicus* resembles *N. ocellatus* in showing no sexual variation in growth rate.

Growth curve data indicate that there is considerable within year variation in adult female body size. This was most evident following maturation. Variation on this scale has been reported in many lizard species (Dunham, 1978; Andrews, 1982; Tilley, 1984; Zug and Rand, 1987; Sinervo, 1990a; Galan, 1996; Olsson and Shine, 1996; Hudson, 1997; Wasptra *et al.*, in press) and is a direct result of high individual variation in growth rate (Andrews, 1982; James, 1991; Smith and Ballinger, 1994a, 1994b; Hudson, 1997). Consequently females show considerable size overlap within age classes once maturity has been reached. Size is therefore a poor indicator of age following maturation and provides little information on age based demographics. Similarly Pengilley (1972) concluded that *Pseudemoia entrecasteauxii* could not be reliable aged beyond one year using size data alone.

Age distribution of mature females (Figure 7.1) was negatively skewed in all but one population. This pattern is expected if age structure is stable (Hudson, 1997). Low altitude populations were most strongly biased towards young animals, indicating that these made up the majority of the population. This may indicate that survival beyond 5 years at these sites is uncommon. At both high altitude sites older animals are seen more commonly. At Mt Wellington, animals appear to easily reach 7 years while the majority of animals continue on until 9 years of age at the Clarence Lagoon site. This has obvious effects on reproductive output within populations. Few animals from low altitude sites appear to live beyond 5 years, and thus are only able to reproduce for 4 years. Animals from high altitude sites can, however, expect to survive well beyond this time frame and even though they mature one year later, they may expect to have reproductive lives of not less than 6 to 7 years. While this type of analysis is not a direct measure of survivorship, the age structures seen to imply that, while high altitude animals display a greater maximum age, mortality pressures during any one year are probably also significantly decreased. Consequently most females at high altitude, that survive an initial winter, can "expect" to live longer lives.

Growth curves produced for *N. metallicus* were created entirely from adult animals, covering the entire body size range observed at each site. Data on small animals may be necessary when fitting asymptotic growth models (Dunham, 1978). This is especially important when discriminating between the von Bertalanffy and logistic models. However, the absence of data on small individuals has a minimal effect on parameter estimates, if

there is sufficient reason to believe *a priori* that the von Bertalanffy model is the correct model to apply (Hudson, 1997). In contrast the absence of data on larger individuals would significantly detract from the power of parameter estimation using this model. Specifically the asymptotic size and growth coefficient estimates would be very unreliable. Hudson (1997) has previously described growth in most members of the *Niveoscincus* and *Pseudemoia* group. In all cases the von Bertalanffy model provided the most satisfactory description of growth.

Minimal differences between growth parameters were evident in *N. metallicus* across the altitudinal range examined. No variation in the asymptotic size or body size at age zero were detected. This trend was also apparent in field observations and other life history data (Chapter 6). The only coefficient to vary notably between populations was that for growth, and this differed significantly only between the two extreme high and low altitude sites (Orford and Mt Wellington). The trend that was evident was for high altitude populations to display a decreased growth coefficient, reflecting an increase in time to reach the asymptotic size. Wapstra *et al.* (in press), in an investigation of *N. ocellatus*, found that all model parameters varied between high and low altitude populations. *Niveoscincus ocellatus* from high altitude displayed significantly greater asymptotic size, birth size and significantly lower growth coefficients. Thus, growth strategies in these two closely related species are quite different. Growth has previously been studied in *N. metallicus* (Hudson, 1997); however, his investigation looked at growth from high altitude populations only, and from mainland and northwestern sub-populations. These populations are genetically distinct from each other and from those sites studied here (Chapter 3), thus making comparative analysis of proximate effects difficult. Hudson also found that these populations were long lived (10 to 11 years), although they matured in only two years. However, other than the significantly increased body size of mainland *N. metallicus* females, growth patterns were not significantly different from those described for the populations I studied.

Few investigations of Australian lizard life histories have examined variation in size and age at maturity on a geographic or altitudinal basis using skeletochronological data. However Rohr (1997), Hudson (1997), and Wapstra *et al.* (in press) provide recent exceptions. In contrast skeletochronology has been used extensively to study geographic variation in age at maturity in amphibians (e.g. Hemelaar, 1988; Bruce and Hairston,

1990; Sagor *et al.*, 1998; Esteban and Sanchiz, 2000; Miaud *et al.*, 2000). Many species of skink mature at a very early age, often within one year (Hudson, 1988; Henle, 1989). However, larger species, or species inhabiting colder climates tend to delay maturity. Some species such as *Tiliqua rugosa*, a large skink, can delay maturity for five years. Greer (1989) reviewed the estimated age of maturity in a number of Australian skinks, but provided minimal data on geographic variability.

Minimum size at maturity was estimated from the smallest female to have given birth or be in late pregnancy from each site. For Hobart this was 47.2 mm, for Orford, 48.1 mm, for Mt Wellington, 47.9 mm and for Clarence Lagoon, 47.7 mm (Chapter 6). Maximum sizes for females from these populations were 61.4 mm, 64.0 mm, 64.2 mm and 62.8 mm respectively. From this it is possible to estimate a ratio of SVL at maturity to maximum female size (Shine and Charnov, 1992). This value has been established as approximately 0.74 in female lizard species (Shine and Charnov, 1992) and ranges from 0.75 to 0.77 in *N. metallicus*. This suggests that size at maturity has been accurately estimated in *N. metallicus*. There is little variation between sites in relation to this variable.

Age at maturity does, however, appear to differ between populations on an altitudinal basis. Animals from both low altitude sites matured at two years of age, while maturation was delayed a further year at high altitude. This is identical to results reported for *N. ocellatus* across a similar altitudinal range. *Niveoscincus ocellatus* from Orford mature at two years, while those from Lake Augusta (comparable to my Clarence Lagoon site) mature at three years (Wapstra *et al.*, in press). However, in contrast, *N. ocellatus* mature at a larger size at high altitude, a phenomenon not observed in *N. metallicus*.

While age and size at maturity are significant life history parameters (Roff, 1992; Stearns, 1992; Galan, 1996; Rohr, 1997), few investigations have examined whether variation in these traits represents proximate environmental influences or genetic adaptation (Adolph and Porter, 1996). Delayed maturity is very common in reptiles inhabiting colder climates (Ballinger and Congdon, 1981; Adolph and Porter, 1993, 1996; Rohr, 1997) and is thought to reflect an inability by these populations to reach minimum maturation size in the time taken by low altitude populations (Adolph and Porter, 1996). Thus *N. metallicus* females from high altitude are only able to achieve minimum reproductive size after three years, whereas those from low altitude require only two years. However, when differences

between thermal opportunity are accounted for (Figure 7.6) it becomes apparent that animals grow at a similar rate between sites. Maturation at identical sizes, but over differing time frames is uncommon in lizards. Generally two patterns are observed. The first, as seen in *N. ocellatus*, is for animals to mature later and at a larger body size at high altitude. This pattern is most common in geographically widespread species (Ballinger and Congdon, 1981; Jones and Ballinger, 1987; Shine and Charnov, 1992; Niewarowski, 1995; Rohr, 1997; Wapstra *et al.*, in press). A second strategy utilized by lizards from colder habitats is to mature at the same age as their warmer site counterparts, but at a significantly reduced size (Forsman and Shine, 1995; Mathies and Andrews, 1995). Among Australian skinks this pattern is best seen in *Lampropholis delicata*, which is significantly smaller in colder southern populations (Forsman and Shine, 1995). The first pattern is generally thought to occur because animals are able to provide additional energy to growth before maturity and thus can obtain increased size in the following year. However, in *N. metallicus* it is apparent that, even given an extra year, high altitude animals can still only just achieve the minimum maturation size found at low altitude. Thus this species appears to follow a third and hitherto undescribed strategy whereby females are unable to gain the advantage of increased size often obtained with delayed maturity.

While age at maturity may be delayed at high altitude, due to limitations in thermal opportunity and possibly in resource availability, the strategy can still be assumed to maximise reproductive output. If delaying maturity resulted in significant costs in future reproduction, then selection could be expected to lead to the evolution of life history adaptations that would maximise lifetime reproductive success (Bernardo, 1993; Adolph and Porter, 1996). However, in cooler environments, mortality pressures, at least in adults, are usually decreased (Adolph and Porter, 1993) due to increased time in hibernation and retreat from inclement weather conditions. Predator numbers may also be decreased at high altitude (Chapter 4). This would result in increased survivorship of age classes from year to year at high altitude, a phenomenon that may be occurring in *N. metallicus* (see earlier discussion). Combined with increased longevity in high altitude populations, and accounting for a slight reductions in clutch size during any one year (Chapter 6), high altitude animals could be expected to display increased future reproductive potential in comparison to low altitude conspecifics (Shine and Schwarzkopf, 1992; Schwarzkopf, 1994). Thus, *N. metallicus* is able to decrease reproductive output at high altitude by

delaying maturity and producing slightly smaller clutches, but is still able to maintain a relatively high lifetime fecundity via increased longevity. *Niveoscincus metallicus* thus appears to only partially fit the models of Adolph and Porter (1993, 1996) as high altitude populations are not characterized by larger adults producing larger clutches.

Skeletochronology proved to be a productive tool for examining age related life-history adaptation in *N. metallicus*. Such studies would be impractical using capture-mark recapture techniques in cryptic and reasonably long-lived species. Differences in growth rates between the high and low altitude populations appear to directly influence age at first reproduction, but not size at first reproduction. Variation in growth rate is believed to directly affect other life-history variables, including size and age at maturity in may lizard species (Grant and Dunham, 1990; Bernado, 1993, Mateo and Castanet, 1994; Smith *et al.*, 1994; Adolph and Porter, 1996; Rohr, 1997; Wapstra *et al.*, in press). However, *N. metallicus* does not follow the same pattern as the closely related *N. ocellatus* in that size at maturity and maximum size are not greater at high altitude. This study supports the view that age at maturity is a phenotypically plastic trait modified by thermal opportunity (Adolph and Porter, 1996; Rohr, 1997) and concludes that *N. metallicus* is able to exploit environments with differing thermal conditions with little if any genetic adaptation being necessary.

CHAPTER EIGHT

General Discussion

Species that occupy broad geographic ranges are presented with significant challenges arising from variation in a number of environmental factors including thermal environment, habitat, food availability, intra and inter specific competition and predation (Smith, 1996, 1998; Schneider *et al.*, 1999). As a consequence, such species often display reproductive, physiological and behavioural traits that compensate for environmental variability (Stearns, 1976; Dunham *et al.*, 1988; Smith *et al.*, 1993; Niewiarowski, 1994). *Niveoscincus metallicus* is found on all but a few offshore islands and on the Tasmanian mainland from sea level to alpine elevations. It also has a more restricted mainland distribution. Across this range the species has been shown to display considerable variation in genotype, as well as in thermal biology and reproductive life history. Previously, Wapstra and Swain (in press) have examined life history adaptation in the ubiquitous sister species *N. ocellatus*. Despite this work, and some other recent Australian research (Forsman and Shine, 1995; Rohr, 1997), information pertaining to variation in any aspect of biology on a geographic basis in southeastern Australian skinks is scant.

Investigations of morphological variation, performance and habitat use in ubiquitous species generally assume that any geographic pattern revealed reflects adaptation to prevailing selective pressures (Losos and Miles, 1994). Similarly, geographic variation in life history characteristics is often assumed to reflect genetic adaptation (Niewiarowski, 1994). However, such interpretations are difficult in the absence of historical information, because phylogenetic effects will confound conventional statistical methods when examining adaptation (Brooks and LcLennan, 1991). The relative importance of geographic isolation versus diversifying selection across environmental gradients, as a force for speciation in reptiles, has been studied in a tropical rainforest setting (Schneider *et al.*, 1999). However, most examinations of ecological adaptation involving analysis of phylogenetic history examine variation between species or genera (e.g. Brooks and McClennan, 1991; Harvey and Pagel, 1991; Melville and Swain, 1998, 2000a). This is because the importance of history is widely appreciated at this level (Niewiarowski, 1994). In contrast, an acknowledgement of the potential influence of history on traits among

populations is largely absent. However, populations, like species and higher taxa, also have an evolutionary history (Avise, 1989), and ignoring that history can affect inferences about the processes responsible for observed patterns of trait variation (Niewiarowski, 1994). While *N. metallicus* has an extensive distribution, adaptation of genotype to local conditions has not occurred in the absence of historical isolation (Chapter 3). Five phylogenetically distinct sub-types were evident across the range of *N. metallicus*. These five groups were identified using both RFLP and sequencing information. Divergence levels indicate that these groups separated during the Pleistocene glaciations. This conclusion is in accord with those of Melville and Swain (1998, 2000a) who proposed that the genus *Niveoscincus* first appeared in the Tertiary, and then diverged in the early Pleistocene. To reduce the effects of historical events in shaping trait variation in modern populations, all field sites for examination of variation in thermal biology and life history were selected from the Type I population. Thus variation observed across this range can be thought to reflect primarily adaptation to prevailing selective pressures.

Lizards can respond in several ways to long term (e.g. seasonal, altitudinal) changes in thermal environment (Van Damme *et al.*, 1989; Spencer and Grimmond, 1994). Behavioural adjustments are generally thought to be the main compensatory mechanism controlling small scale spatial and temporal variation in a thermal environment (Hertz and Huey, 1981; Avery, 1982; Van Damme *et al.*, 1987). These small scale behavioural adaptations generally take the form of shifts in basking, activity times, postural changes and microhabitat selection. Daily and seasonal activity patterns have been examined in high altitude *N. metallicus* from Mt Wellington (Melville and Swain, 1997b). These animals were found to be diurnally active. Activity was not examined in my investigation, although variation in both seasonal and daily activity may be expected between sites. Microhabitat occupation was, however, investigated as a part of this study (Chapter 4), and appears to represent a significant aspect of thermal control in *N. metallicus*. The most obvious response observed was that high altitude animals tend to bask more overtly and generally on rock, in order to maintain a constant body temperature. In contrast animals at low altitude generally bask under cover and on wood.

Behavioural adaptations can, however, represent an inadequate means of compensating for differing thermal loads, or they may be too costly in terms of time and energy (Huey and Slatkin, 1976). Under such conditions a lizard may display some level of physiological

acclimatization and/or genetic adaptation to varying environmental conditions (Van Damme *et al.*, 1989). *Niveoscincus metallicus* shows strong evidence for genetic adaptation across its altitudinal range. This was most obvious from thermal limit differences evident between high and low altitude populations; these were not affected by laboratory acclimation and were also present in newborns (Chapter 5). In contrast, small seasonal fluctuations within populations were the only evidence of any acclimatization effects. Data from *N. metallicus* support the "labile" view of thermal adaptation in reptiles, despite the fact that body temperature is effectively maintained at a similar level at all sites. Interestingly *N. metallicus* also displayed variation in the thermal relations of their sprint performance with high altitude animals sprinting significantly faster at extreme temperatures. Thus, curve breadth but not relative position was affected by altitude. This pattern is, to the best of my knowledge, unreported in any other lizard. Both Van Damme *et al.* (1989) and Crowley (1985) supported the "static" view in examinations of sprint curves between thermally distinct populations of *Podarcis tiliguerta* and *Sceloporus undulatus* respectively. *Niveoscincus metallicus* also showed altitudinal variability in willingness to sprint, with high altitude populations stopping less often at extreme temperatures.

Niveoscincus metallicus therefore appears to use behavioural mechanisms to enable it to maintain T_b at a similar level at all sites. However, at high altitude animals will often be unable to maintain a high T_b, or will require significant time to achieve a preferred T_b. As high altitude lizards are often exposed on rocks and are less wary (personal observation), increased sprinting speed and willingness to sprint at low T_bs may represent a significant adaptation to cooler environments. Sprint speed has been shown to scale directly with foraging and escape success (Huey and Stevenson, 1979; Christian and Tracy, 1981). Thus, as high altitude populations must spend significant periods of time at T_bs that are considerably lower than those preferred, selection should favor a shift in thermal limits and performance to adapt to cool conditions. This should be most evident during spring and autumn. The fact that *N. metallicus* is restricted to an annual reproductive cycle, and collection of body reserves primarily occurs during spring and autumn in females, means that animals must be active during these periods even if environmental conditions are poor. The Tasmanian environment is very unpredictable, with high altitude sites periodically remaining cold until at least November and animals often forced to retreat as early as April. Snow may also fall at any stage of the year, even during mid summer. As a

result the ability to be active and able to forage during cool periods would represent a significant adaptation and may be one factor that allows high altitude populations to maintain an annual reproductive cycle, even during very poor years.

In contrast to thermal adaptations, which appear to have a significant genetic component, adaptation of life history characteristics seems to occur primarily through proximate variation induced by environmental constraints. What induces variation in life history characteristics, either genetic variation or plastic responses to environment, represents a central question in modern biology. It has become apparent that many patterns of geographic variation among lizard populations are induced primarily by proximate factors such as thermal conditions and food availability (Roff, 1992; Adolph and Porter, 1993, 1996; Abell, 1999; Wapstra and Swain, in press); however, genetic variation between populations has also been demonstrated (Niewiarowski and Roosenburg, 1993). An understanding of the role of proximate influences on variation in life history characteristics is important for a complete understanding of life history patterns (Ballinger, 1983; Sinervo and Doughty, 1996).

Variation in life history characteristics in *N. metallicus* was found at both a geographic level and, to a lesser extent, at an annual level within populations. Annual variation within a population can only result from phenotypic responses to environmental variability; however, geographic variation can have both proximate and genetic components. Little intra-annual variability was observed in *N. metallicus*, although proximate effects have been shown to influence reproductive output and neonate size in *N. metallicus* (Swain and Jones, 2000b). Clutch size was observed to vary on an annual basis, as did some aspects of offspring morphology and fat body deposition. Thus, all aspects of maternal biology appeared to be capable of responding to proximate influences. Nevertheless, variation on a geographic scale was more pronounced, specifically in terms of RCM which was reduced at high altitude (Chapter 6). Thus the possibility exists that genetic adaptation is present in this species. The occurrence of genetic variation in thermal biology in *N. metallicus*, despite its general lack of importance in most species, further supports the conclusion that genotype may play a role in maintaining reproductive variability between populations in this species.

In contrast to the above life history characteristics, growth, age at maturity and longevity were all clearly under the control of proximate influences (Chapter 7). Size at maturity and maximum size did not differ between populations. The life history pattern exhibited by *N. metallicus* at high and low altitude is reasonable consistent with the models proposed by Adolph and Porter (1993, 1996). These models propose that variation in life history between populations can be explained in terms of the effects of the thermal environment on daily and seasonal activity. They predict that animals from populations subject to short potential activity seasons (high altitude) will have a high annual survival, a low reproductive effort, delayed maturity, and maturity will occur at a large size. In contrast animals from sites with long activity seasons (low altitude) will display early maturity at a smaller size, greater reproductive effort, and a lower annual survival rate. *Niveoscincus metallicus* conforms to this model in most respects, the exception being that size at maturity does not differ between sites. Reproductive output did conform to predictions in *N. metallicus*, although, in the sister species, *N. ocellatus*, RCM did not change in populations from high and low altitude (Wapstra and Swain, in press). Further work is required to better understand the factors that influence reproductive output between populations of skinks, because to date no consistent patterns have emerged. In addition to the work described here on *Niveoscincus* species, Forsman and Shine (1995) found increased reproductive output in populations with shorter activity seasons in *Lampropholis delicata*, whereas in *Eulamprus tympanum*, RCM followed a pattern similar to that found by me for *N. metallicus* (Rohr, 1997).

The characteristics of offspring phenotype that were measured produced generally consistent results both within and between field sites (Chapter 6). The only exceptions to this generalisation were young from Orford which were larger than neonates from other sites and offspring from high altitude which had significantly heavier fat reserves than those from low altitude populations. Variation in size of offspring is often explained in terms of selection pressures at specific sites. While this is undoubtedly true in *N. metallicus*, there also appears to be a trade-off occurring between offspring size and number in low altitude populations. RCM at low altitude is high; however, it is suggested that predation appears to select for large offspring at the Orford site. As a result, perhaps because of space limitation (Olsson and Shine, 1997) females reduce the number of young they carry. Within population trade-offs between offspring size and number were not examined in this thesis. The other characteristic that differed between populations in *N.*

metallicus was fat reserves at birth. Larger fat reserves at high altitude have obvious advantages. Embryos at high altitude can use these reserves to maintain themselves at stage 40 if external conditions delay birth, and young may use these reserves to survive their first winter, which arrives no more than two months after birth. In contrast, low altitude young have about 4 months to collect resources before they overwinter. Whether genetic or environmental factors promote this extra fat deposition is unclear; however, Swain and Jones (2000b) suggest that proximate control is significant, with environmental conditions influencing neonate fat reserves via facultative placentotrophy in *N. metallicus* (Swain and Jones, 2000a). To date no other research has examined variation in fat levels in newborn offspring on a geographic scale.

Swain and Jones (2000b) have shown that thermal environment and food availability during gestation directly affect offspring phenotype and fat reserves in *N. metallicus*. Data from the Orford site also indicate that fat reserves can vary significantly on an inter-annual level within a site. Since food quantity and thermal conditions available to pregnant females are generally good at low altitude, females would have the resources required to provide young with additional abdominal fat stores. However, RCM is significantly lower in high altitude populations. As a result, females from these sites may be able to eat prey even during late pregnancy, while low altitude mothers are unable to ingest significant amounts of food. Indeed decreased food intake in low altitude mothers was observed in the laboratory (personal observation) and has been reported as a significant cost of reproduction in reptiles (Schwarzkopf, 1996). Female *Eulamprus tympanum* with relatively large numbers of ova or embryos ate relatively less, and therefore decreased food intake in females may represent a fecundity-dependent cost of reproduction in some lizards. Thus, an ability to hold more food and the fact that mothers must support a smaller litter may provide a female with excess reserves, which can be redirected to offspring reserves, through facultative placentotrophy. This may provide another strong selective advantage of decreased RCM at high altitude, further supporting the possibility of a genetic component to this trait in high altitude populations. What is apparent is that offspring morphology and fat reserves, while both obviously affected strongly by the same proximate forces, act independently of each other in *N. metallicus*.

Despite the evidence of some degree of genetic variation in *N. metallicus*, flexibility in life history, reproduction and habitat use all play a major role in allowing this species to

maintain it widespread distribution. Reproductive events are delayed at high altitude and this strategy ensures that gestation occurs relatively rapidly during the warmest months at these sites. Other widespread species also display this characteristic (e.g. *Anolis sagrei*, Lee *et al.*, 1989; *Sceloporus scalaris*, Mathies and Andrews, 1995; *N. ocellatus*, Wapstra and Swain, in press). Similarly relatively precise thermal control of T_b, as is evident in *N. metallicus*, requires some flexibility in the use of structural habitat elements (Christian *et al.*, 1983; Adolph, 1990). *Niveoscincus metallicus* also displays considerable life history plasticity in responses to environmental conditions, particularly temperature. This plasticity in the face of varying environmental conditions represents a significant advantage to species living across large ranges and/or in fluctuating environments. Widespread species that are not genetically restricted in their phenotypic responses may be better able to cope with temporal climatic variation, thus further enhancing their fitness. Thus, the evolution of distinct local genotypes in fluctuating environments may not occur if phenotypic plasticity is sufficient to induce variation in traits to suit that environment.

Field studies examining correlation between environmental conditions and aspects of an organism's ecology are important to our understanding of geographic variability in factors such as life history (Ballinger, 1983). However, the number of uncontrolled variables in such studies makes identification of causation difficult. *Niveoscincus metallicus* provided an excellent model to investigate adaptation on a geographic scale using largely field data. This species appears to follow a somewhat uncommon pattern in terms of thermal adaptation, with genetic factors playing a pivotal role in survival at high altitude. In contrast, it adopts a more conventional approach to life history adaptation, opting for a largely environmentally induced variability; however, some aspects of life history may still be controlled on a genetic basis. A logical extension of this study would be to examine, using common garden or transplant design experiments, the genetic versus environmental importance of variation in offspring fat deposition between high and low altitude populations. Also variation in RCM between sites could be examined; however, this would require growing juveniles under common conditions until adult size (2 years) and is probably unfeasible as a short term study. Most studies examine the genetic versus environmental importance to growth in neonates (Niewiarowski and Roosenburg, 1993; Sorci *et al.*, 1996; Wapstra, 1988). However, growth variation in most species, including *N. metallicus*, is found to result primarily from proximate sources. Thus, other life history

variables need to be examined to truly understand the importance of genetic factors on life history adaptation.

CHAPTER NINE

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